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Draft Criteria Document for Carbon Tetrachloride

JRB Associates, Inc., McLean, VA

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This document summarizes the health effects of Carbon Tetrachloride. Topics include: General Information and Properties; Human Exposure; Pharmacokinetics; Health Effects in Animals and Humans; Mechanisms of Toxicity; Risk Assessment and Quantification of Toxicological Effects.

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PREFACE

The objective of this criteria document is to assess the available data on health effects associated with exposure to carbon tetrachloride in drinking water and to recommend a maximum contaminant level. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic health effects in animals and humans, mechanisms of toxicity were evaluated. Only the reports which were considered pertinent for the derivation of the maximum contaminant level are cited in the document. Particular attention was paid to the utilization of primary references for the assessment of carbon tetrachloride-induced health effects. Secondary references were used rarely. For comparison purposes, standards and criteria developed by other organizations are included and discussed in Section IX (Quantification of Toxicological Effects of Carbon Tetrachloride).

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*Prepared by the Science and Technology Branch

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I. SUMMARY

Carbon tetrachloride, also known as tetrachloromethane and perchloromethane, is a haloalkane with a wide range of industrial and chemical applications. In 1980, 322,000 kkg were produced in the United States. Most of the chemical produced is used in the manufacture of fluorocarbons, which are used as refrigerants, foam blowing agents, and solvents. Carbon tetrachloride is also used as a solvent in metal cleaning and in the manufacture of paints and plastics as well as in fumigants. It is being largely replaced in grain fumigation by other registered pesticide products.

Inhalation is the most important route of carbon tetrachloride exposure. There are no major gradients in the atmospheric distribution of carbon tetrachloride; the concentrations are similar in the continental and marine air masses ambient (approximately 0.00070-0.00084 mg/m^3 or 111-133 ppt). The maximum level of carbon tetrachloride reported was 0.113 mg/m^3 (18,000 ppt) in Bayonne, New Jersey.

Carbon tetrachloride has been found in many sampled waters (including rain, surface, potable, and sea) at the ppb level. The National Organics Monitoring Survey (NOMS) sampled 113 public water systems and found carbon tetrachloride at very low concentations, relative to levels of chloroform and other organics. Positive results were noted in about 10 percent of the samples, with mean values ranging from 2.4-6.4 ppb depending on sampling and analytical procedures. An accidental discharge of an estimated 70 tons of carbon tetrachloride into the Kanawha River resulted in levels of carbon tetrachloride as high as 300 ppb in raw Ohio river water; the drinking water levels were found to be as high as 100 ppb. Chlorination of raw waters with chlorine gas contaminated with carbon tetrachloride may account for the presence of the compound in some finished drinking waters.

Carbon tetrachloride has been detected in a variety of foodstuffs other than fish and shellfish in levels ranging from 1-20 ppb. Carbon tetrachloride contaminations of food categories has been reported as follows: Dairy products (0.2-14.0 ppb); meat (7.0-9.0 ppb); oils and fats (0.7-18.0 ppb); beverages (0.2-6.0 ppb); fruits and vegetables (3.0-8.0 ppb); black grapes (19.9 ppb); and fresh bread (5-10 ppb). The amount of carbon tetrachloride residue in some foodstuffs depends on the fumigant dosage, storage conditions, length of aeration, and extent of processing.

No information was found on levels or frequenc~~ies~~ of human dermal exposures. Carbon tetrachloride is no longer on the market as a component of hair shampoo or cleaning solvents, and because of strict OSHA safety regulations, dermal exposure in the workplace has been minimal.

Carbon tetrachloride is absorbed into circulation through the lungs and the skin. Absorption from the gastrointestinal tract also readily occurs but at a slower rate. Following a single exposure, high concentrations of carbon tetrachloride were detected in the blood, brain, kidney, and liver. With repeated exposures, carbon tetrachloride is present mostly in the liver and, because of its lipophilic nature, in the body fat and bone marrow.

In both animals and humans exposed to carbon tetrachloride, the liver is the first organ to be affected. Minor liver damage, as evidenced by increased levels of liver enzymes in the serum, progresses through early necrotic changes, increased vacuolization and fat droplets, to necrosis, degeneration, and fatty liver. Most acute liver damage is reversible with time. Chronic necrotic and degenerative changes, however, appear not to be reversed as readily. Exposure to other hepatotoxins especially ethanol and drugs such as barbiturates, greatly exacerbates the hepatic toxicity of carbon tetrachloride. Pulmonary damage occurs, but mostly at an ultrastructural level and to a much lesser extent than liver damage. Renal and pancreatic effects also have been observed, but only following massive doses of carbon tetrachloride.

In humans, acute toxic effects of carbon tetrachloride include gastrointestinal disturbances, nausea, and vomiting. Hepatic injury occurs with all of the signs of severe cellular damage. Serious renal injury usually does not become evident until 24-48 hours or several days after the onset of initial symptoms of poisoning. In severe cases, death from hepatic coma and uremia occurs within 1-2 weeks. After repeated or prolonged inhalation of less toxic concentrations of carbon tetrachloride, gastrointestinal and central nervous system symptoms occur such as nausea, vomiting, visual disturbances, and giddiness. After repeated exposure, severe hepatorenal damage can occur. Treatment and cessation of exposure usually result in disappearance of toxic symptoms and reversal of the liver and kidney damage.

Carbon tetrachloride has been shown to be carcinogenic in rats, mice, and hamsters. Although a number of cases of liver tumors following exposure to carbon tetrachloride have been reported, the chemical has not been established to be a human carcinogen. Although it is not mutagenic in the Salmonella (Ames) assay, carbon tetrachloride has elicited a mutagenic response in the yeast Saccharomyces cerevisiae. Carbon tetrachloride has also been shown to be fetotoxic and to inhibit male fertility in rats.

The mechanism of toxicity of carbon tetrachloride has been extensively studied with special emphasis on the hepatic effects. Carbon tetrachloride is metabolized by the liver mixed function oxidase system to trichloromethyl (and other) free radicals, which can either react with hepatic macromolecules or be further metabolized via chloroform and phosgene to carbon (mono/) dioxide. The reaction of the trichloromethyl radical with cellular lipids initiates a lipid peroxidation resulting in the progressive and exponential destruction of membranes. Membrane permeability also is greatly increased as a result of lipid peroxidation. Although some investigators still maintain that direct reaction with hepatic macromolecules result in hepatic damage, the recent ultrastructural evidence tends to substantiate the lipid peroxidation theory.

By extrapolation from animal studies, the National Academy of Sciences (NAS) and EPA's Carcinogen Assessment Group (CAG) have calculated projected incremental excess cancer risk associated with the consumption of specific chemicals in drinking water over a 70-year lifetime. Using the multistage model, NAS estimated that comsumption of 2 liters of water per day over a lifetime at carbon tetrachloride concentrations of 450, 45, or 4.5/liter would result in the induction of one excess case of cancer per 10,000, 100,000, or 1,000,000 people exposed, respectively.

Using the "improved" multistage model, CAG estimated that consumption of 2 liters of water per day at carbon tetrachoride concentrations 42.2 ug/l, 4.2 ug/l, or 0.4 ug/l would result in the induction of one excess case of cancer per 10,000, 100,000, or 1,000,000 people exposed, respectively.

The differences between NAS and CAG risk estimates are partly explained by the fact that the extrapolation models used by the two groups, although similar, are not identical. The NAS risk estimate was arrived at using the multistage model; whereas, the CAG derived their risk estimate using the "improved" version of the multistage model. In addition, the models also differ in the data selected and other parameters. The NAS model used rats as the comparison species; the CAG model used mice.

These modeling methods share the assumption that there is no threshold level for the action of a carcinogen. However, no one method can accurately predict the absolute numbers of excess cancer deaths that will be attributable to carbon tetrachloride in drinking water. None of the methods presently used to quantify carcinogenic risk accounts for increased carcinogenic risk from the interaction of carbon tetrachloride with other environmental contaminants to which humans can be exposed.

It is noteworthy that in assessing CCl_4-induced toxicity, carcinogenicity or any other harmful effect, compounds that react synergistically or antagonistically with CCl_4 must be considered. Identified synergistic substances include ethanol, kepone, PCB, and PBB. Antagonistic effects have been demonstrated with such compounds as chloramphenicol and catechol. Sensitive populations are subgroups within the general population which appear at higher than average risk upon exposure to CCl_4. Some of the populations that may be at greater risk include human fetuses, alcohol consumers, and males of reproductive age.

The quantification of non-carcinogenic effects in humans could not be undertaken at this juncture due to lack of acceptable chronic exposure data for this compound. Because of positive results in animal carcinogenicity studies, carbon tetrachloride can be considered a suspect human carcinogen. Thus, the recommended maximum contaminant level (RMCL) for carbon tetrachloride should be based on its carcinogenic potential. According to the Safe Drinking Water Act (42 USC 300F, SDWA, 1974), this level should be zero (0 mg/L).

II. GENERAL INFORMATION AND PROPERTIES

Carbon tetrachloride, or tetrachloromethane, is a colorless liquid with a molecular weight of 154 and a boiling point of 76.5°C (Weast, 1972). It is a relatively nonpolar compound that is miscible in alcohol, acetone, and other organic solvents (Weast, 1972), but is only minimally soluble in water (0.8 g/liter at 25°C) (Johns, 1976). The octanol/water partition coefficient of carbon tetrachloride is 2.64 (Johns, 1976).

The properties of carbon tetrachloride favor volatilization of the compound from water to air. Carbon tetrachloride has a high vapor pressure (115.2 mm Hg at 25°C) (Johns, 1976). The air/water partition coefficient of carbon tetrachloride at 20°C is 1.1 by volume, and about 1,000 by weight (Johns, 1976). The rapid vaporization predicted from these properties has been confirmed by Dilling *et al*. (1975), who reported a half-life of carbon tetrachloride evaporation of 29 minutes from a dilute aqueous solution at about 25°C.

The density of carbon tetrachloride is 1.59 g/ml at 20°C (Weast, 1972). Because its density is greater than the density of water, some carbon tetrachloride from large spills in water might tend to settle before it is totally dispersed, emulsified or volatilized.

Carbon tetrachloride is produced industrially by the chlorination of methane, propane, ethane, propylene, or carbon disulfide (Rams *et al.*, 1979). In 1980, 322,000 kkg were synthesized (USITC, 1981). Carbon tetrachloride is also produced indirectly during the production of compounds such as vinyl chloride and perchloroethylene (Rams *et al.*, 1979).

The major use of carbon tetrachloride is in the production of chlorofluorocarbons, which are used as refrigerants, foam-blowing agents, and solvents. Carbon tetrachloride is also used in fumigants, and has a variety of minor uses, including those as a solvent in metal cleaning and in manufacture of paints and plastics (Rams *et al.*, 1979). It is being replaced in grain fumigation by other registered pesticide products (USEPA, 1980a), and its registration for use in fumigants is presently under review by USEPA (USEPA, 1980b).

Carbon tetrachloride present in the environment appears to be of anthropogenic origin (Singh *et al.*, 1976). It can enter natural waters through industrial and agricultural activities. Carbon tetrachloride may be carried to surface waters through run-off from agricultural, industrial, and dumping sites, and through industrial effluents. Industrial emissions may also contribute carbon tetrachloride to the air, from which the compound may reach surface water through rainfall. Carbon tetrachloride may also reach groundwater through leaching from solid waste sites.

Once in the environment, carbon tetrachloride is relatively stable. Its half-life for hydrolytic breakdown in water at pH 1.0-7.0 is estimated to be 70,000 years, but hydrolysis appears to be accelerated in the presence of metals such as iron and zinc (Johns, 1976). The high stability in water has little practical significance; however, since carbon tetrachloride vaporizes readily to air. The atmospheric lifetime of carbon tetrachloride appears to be on the order of 30-100 years (Singh *et al*., 1976).

The presence of carbon tetrachloride in the environment is of concern for two reasons. First, carbon tetrachloride may contribute to ozone-destroying photochemical reactions in the stratosphere, which might cause increases in the incidence in human skin cancers and animal cancers, affect terrestrial and aquatic ecosystems, and bring about climatic changes (NAS, 1978).

Although levels of carbon tetrachloride in the environment are generally in the low ppb range or below (NAS, 1979), this chemical may pose a long-term danger because of its possible carcinogenic potential. In urban and industrial areas where higher concentrations of carbon tetrachloride occur, other toxic effects may result (e.g., liver and renal damage). The following chapters discuss the evidence for health effects attributed to carbon tetrachloride, with particular emphasis on ingestion via drinking water.

Analysis of Carbon Tetrachloride in Drinking Water

Carbon tetrachloride (and 47 other halogenated organics) in water can be analyzed by a purge and trap method (Method 502.1) described by the EPA Environmental Monitoring and Support Laboratory (USEPA, 1980d). This method can be used to measure purgeable organics at low concentrations. Purgeable organic compounds are trapped on a Tenax GC-containing trap at 22°C using a purge gas rate of 40 ml/min for 11 minutes. The trapped material is then heated rapidly to 180°C and backflushed with helium at a flow rate of 20-60 ml/min for 4 minutes into the gas chromatographic analytical column. The programmable gas chromatograph used is capable of operating at 40°±1°C. The primary analytical column is stainless steel packed with 1% SP-1000 on Carbopack B (60/80) mesh (8 ft x 01 in. I.D.) and is run at a flow rate of 40 ml/min. The temperature program sequence begins at 45°C for 3 minutes, increases 8°C/min to 220°C, and is then held constant for 15 minutes or until all compounds have eluted.

A halogen-specific detector with a sensitivity to 0.10 ug/ liter a relative standard deviation of 10% must be used. The optional use of GC/MS techniques of comparable accuracy and precision is acceptable.

Treatment of Carbon Tetrachloride in Drinking Water

The information available on the removal of carbon tetrachloride from drinking water is limited. However, as judged from data obtained for industrial waste treatment, conventional treatment processes are not very effective in the removal of this compound. An isotherm study of carbon tetrachloride on Filtration 400 activated carbon (GAC) showed that at an equilibrium concentration range of 3 x 10^{-9} to 2.6 x 10^{-7} mol/liter, a maximum surface concentration of 2.6 x 10^{-5} mol/g was obtained (NAS, 1979). Aeration and adsorption processes have also been evaluated for removal of this compound. Powdered activated carbon (PAC) at 2 to 4 mg/liter was not effective in treating contaminated river water containing 16.3 mg/liter of carbon tetrachloride. After PAC, coagulation, settling, and filtration, the finished water still contained 16.0 mg/liter (USEPA, 1980d). Aeration by diffused air aerator in a laboratory study was found to be more successful. At 4:1 air-to-water ratio, a 91 percent removal efficiency for carbon tetrachloride was achieved (USEPA, 1980d). Adsorption by GAC in a pilot scale study revealed that carbon tetrachloride at an average concentration of 12 mg/liter (Cincinnati tap water) was reduced to less than 0.1 ug/liter for 3 weeks with a 5-minute empty bed contact-time (EBCT) and for 14-16 weeks with a 10-minute EBCT.

Summary

Carbon tetrachloride is a colorless liquid at ambient temperature. Its high vapor pressure favors rapid volatilization from water to air. Carbon tetrachloride is produced commercially from the chlorination of methane, propane, ethane propylene or carbon disulfide and its major use is in the production of chloroflurocarbons. Carbon tetrachloride is present in the enviroment by anthropogenic means and once in the environment appears relatively stable. Its presence is of concern because of a possible contribution to ozone destroying chemical reaction in the atmosphere and because ingestion via drinking water may present a human health hazard. Carbon tetrachloride in water may be detected by the purge and trap method described by the USEPA Enviromental Monitoring and Support Laboratory. Removal of this chemical from water may proceed by aeration and filtration through appropriate media.

III. PHARMACOKINETICS

Absorption

Partition Coefficients. Partition coefficients for various chlorinated solvents, including carbon tetrachloride, were determined by several experimenters (Morgan *et al*., 1972; Sato and Nakajima, 1979; Powell, 1945). The partition coefficient is a measure of the relative solubility of a substance in two media. The oil/air and oil/water partition coefficients can be used as indicators of solubility in lipids. The values of these and other partition coefficients for carbon tetrachloride, listed in Table III-1, show this chemical to be lipophilic. Because of its lipophilic nature, one would predict that carbon tetrachloride, could be absorbed by ingestion, inhalation, and skin contact. This prediction is borne out by results of the experimental studies described below.

TABLE III-1 Partition Coefficients for Carbon Tetrachloride

	K_D		
Parameter	20°C	25°C	37°C
Olive oil/air		142	
Olive oil/air			361
Blood serum/air		6	
Blood/air			2.4
Blood/air	3.6-5.2		
Water/air		0.25	
Olive oil/water		1,440	
Olive oil/serum		23	
Olive oil/blood			150

Adapted from Morgan *et al*. (1972), Sato and Nakajima (1979), and Powell (1945).

Absorption from the Gastrointestinal Tract. Absorption of carbon tetrachloride through the gastrointestinal tract of dogs was studied by Robbins (1929). In a series of experiments, the author determined the amount of carbon tetrachloride absorbed from the ligated stomach, small intestine, and colon by measuring the concentration of carbon tetrachloride found in the exhaled breath. The greatest concentration of carbon tetrachloride in exhaled air was seen after injection of the chemical into the small intestine. Direct injection of carbon tetrachloride into the colon resulted in a lower concentration of the chemical in exhaled air. After introduction directly into the stomach by intubation, no carbon tetrachloride was detected in exhaled air. The method of detection in these experiments was thermal conductivity, with stated detection limits of one part in ten. Thus, the results of the experiment can be viewed as a qualitative indication of relative absorption from the various components of the gastrointestinal tract, rather than as quantitatively accurate results.

Absorption by Inhalation. Von Oettingen *et al.* (1950) studied the absorption of carbon tetrachloride by inhalation in Beagle dogs. The sex was unspecified, but the authors stated that at least five dogs were used in each experiment. The dogs inhaled carbon tetrachloride (purity unspecified) at a concentration of 94,500 mg/m^3 (15,000 ppm) for 475 minutes through a two-way valve attached to the cannulated trachea. Blood samples were taken at unspecified intervals and analyzed for carbon tetrachloride. Data presented graphically showed that the concentration of carbon tetrachloride in blood reached a maximum of 31.2-34.3 mg/100 cc (0.20-0.22 millimole percent) after approximately 300 minutes of exposure and remained at that level for the duration of the exposure.

McCollister _et al_. (1951) investigated the absorption of carbon tetrachloride by inhaltion using rhesus monkeys. Three female monkeys inhaled 99.9% ^{14}C-labeled carbon tetrachloride at an average concentration of 290 mg/m^3 (46 ppm) for 139, 344, and 300 minutes, respectively. The authors calculated by difference between inhaled and exhaled air that the monkeys absorbed an average of 30.4% of the total amount of carbon tetrachloride inhaled. Analysis of blood drawn after 270 minutes of exposure showed that the ^{14}C radioactivity was equal to 0.297 mg of carbon tetrachloride/ 100 g of blood, distributed as follows: 56.2% as carbon tetrachloride, 16.5% as "acid-volatile" carbonates, and 27.3% as nonvolatile material. No attempt was made to characterize metabolites in this study.

Absorption Through the Skin. McCollister et al. (1951) exposed the skins of one male and one female rhesus monkey to ^{14}C-labeled carbon tetrachloride vapor. To determine the amount of absorption, blood and exhaled air were analyzed for ^{14}C radioactivity. After a skin exposure of 240 minutes to carbon tetrachloride vapor at 3,056 mg/m^3 (485 ppm), the blood of the female monkey contained carbon tetrachloride at 0.012 mg/100g and the exhaled air contained 0.0008 mg/liter. After exposure to 7,345 mg/m^3 (1,150 ppm) for 270 minutes, blood of the male monkey contained carbon tetrachloride at 0.03 mg/100g and the exhaled air contained 0.003 mg/liter.

Three human volunteers, sex unspecified, immersed their thumbs in carbon tetrachloride for 30 minutes in an experiment to measure skin absorption (Stewart and Dodd, 1964). Carbon tetrachloride was analyzed by infrared spectroscopy and was found to contain no detectable impurities. The concentration of carbon tetrachloride in alveolar air was used as the indicator of absorption and measured at 10, 20, and 30 minutes after the start of exposure and at 10, 30, 60, 120, and 300 minutes after cessation of exposure. Carbon tetrachloride was present in the alveolar air at each time interval, reached a maximum concentration range of 2.8-5.7 mg/m^3 (0.45-0.79 ppm) 30 minutes after exposure, and decreased exponentially thereafter. The authors concluded that carbon tetrachloride could be absorbed by the skin in toxic amounts if the chemical came in contact with arms and hands.

Distribution

Robbins (1929) administered 159 g (100 cc) of carbon tetrachloride, purity unspecified, to three anesthetized dogs by stomach tube. The dogs were sacrificed at 6, 23, and 24 hours after treatment and blood and various tissues were analyzed for carbon tetrachloride by converting the organic chloride to inorganic chloride and titrating the inorganic chloride by the Volhard method, which is accurate to 0.1-0.2%. The results of the blood and tissue analysis are shown in Table III-2.

TABLE III-2 Carbon Tetrachloride Distribution at Various Times After Administration by Stomach Tube (mg/100 g of tissues)

Tissue	6 hrs	23 hrs	24 hrs
Brain	--	17	9
Blood, portal	26	13	22
Blood, arterial	0	0	0
Bone marrow	--	66	--
Kidney	--	11	13
Liver	15	10	27
Lungs	--	trace	6
Muscle	--	trace	20
Pancreas	--	4.5	14
Spleen	--	5	--

From the experimental data, it appears that the limit of detection was in the range of 4-5 mg of carbon tetrachloride/ 100 g of tissues. In addition, it appears that the liver, bone marrow, blood, and muscle retained the most carbon tetrachloride for the longest time.

Von Oettingen et al. (1950) reported the tissue distribution of carbon tetrachloride in Beagle dogs, each weighing about 10 kg, exposed to carbon tetrachloride in air at 94,500 mg/m^3 (15,000 ppm) for 475 minutes. The dogs were sacrificed at the end of the exposure. Tissue and blood samples were taken and analyzed for carbon tetrachloride. The concentration of carbon tetrachoride (per 100 g of tissue) was 66 mg/100 g in the brain, 50 mg/100 g in the heart, 36 mg/100 g in the liver, and 34 mg/100 g in the blood; the concentratiion in the fat was not determined. The investigators stated that the accumulation of carbon tetrachloride in the brain was consistent with its high oil/water partition coefficient and resulted in its strong narcotic action.

McCollister et al. (1951) reported the tissue distribution of carbon tetrachloride in rhesus monkeys exposed to 290 mg/m^3 (46 ppm) of [^{14}C] carbon tetrachloride for 300 minutes. The tissue distribution, as calculated from the ^{14}C radioactivty, is shown in Table III-3. The concentration of carbon tetrachloride was greatest in the fat, followed by the liver and bone marrow.

TABLE III-3 Tissues Distribution of [^{14}C] Carbon Tetrachloride Inhaled by Rhesus Monkeys

Tissue	Carbon tetrachloride (mg/100 g of tissue)
Fat	2.46
Liver	0.94
Bone marrow	0.93
Blood	0.31
Brain	0.30
Kidney	0.23
Heart	0.14
Spleen	0.10
Muscle	0.06
Lung	0.04
Bone	0.04

Souce: McCollister _et_ _al_. (1951)

Fowler (1969) studied the distribution of carbon tetrachoride in the tissues of rabbits given the chemical by stomach tube. Five rabbits were given carbon tetrachloride

(1 ml/kg bw) as a 20% (v/v) solution in olive oil. Analysis of the carbon tetrachloride by gas chromatography showed not more than 125 ppb of hexachloroethane. The rabbits were sacrificed 6, 24, and 48 hours after treatment and the tissues analyzed for carbon tetrachloride by gas chromatography equipped with an electron capture detector. Six hours after carbon tetrachloride was administered, the tissue concentrations (per kg of tissue) were 787 ± 289 (Mean ± SEM) mg/kg in fat, 96 ± 11 mg/kg in liver, 21 ± 12 mg/kg im muscle, and 20 ± 13 mg/kg in kidney. By 48 hours, these concentrations had dropped to 45 ± 12 mg/kg in fat, 4 ± 0.1 mg/kg in liver, and 0.5 ± 0.3 mg/kg in kidney and muscles. These data indicate that most of the dose of carbon tetrachloride is eliminated from rabbit tissues within 48 hours.

Metabolism

Chloroform was one of the first carbon tetrachloride metabolites to be described (Butler, 1961). Eight dogs were exposed to carbon tetrachloride by tracheal cannula at the rate of 8,000 mg/hr for 3 hours. At the cessation of exposure, the exhaled air from the dogs was collected and analyzed by both gas chromatography and the Fujiwara reaction, a colorimetric procedure for the identification of chloroform. Chloroform was detected in the exhaled breath by both of these methods. The total amount of chloroform exhaled in 2 hours by each dog was estimated at 0.10.5 mg by analysis of gas chromatographic data. Tissue homogenates were also shown to metabolize carbon tetrachloride to chloroform.

Evidence of metabolism to a free radical was suggested by studies showing hexachloroethane to be a carbon tetrachloride metabolite (Bini _et_ _al_., 1975). Five Wistar rats were administered 160-800 mg of carbon tetrachloride diluted in liquid paraffin by gavage following a 24-hour fast. The animals were sacrificed 15 minutes to 8 hours after treatment. A graph displaying carbon tetrachloride concentrations in rat liver versus time showed the chemical at approximately 0.9 mg/kg of tissue after 15 minutes and at maximal concentration (1.7 mg/kg) after 120 minutes. Analysis of the gas chromatographic data showed that chloroform, was maximal at 0.037 mg/kg after 15 minutes; after 4 hours it had declined to 0.007 mg/kg. Hexachloroethane was also present after 4 hours 0.005 mg/kg. The authors explained the formation of both chloroform and hexachloroethane as carbon tetrachloride metabolites by proposing that the trichloromethyl free radical was the primary metabolite of carbon tetrachloride.

^{14}C-labeled carbon dioxide was detected in the exhaled air of rhesus monkeys after a 344-minute exposure to

$[^{14}C]$ carbon tetrachloride by inhalation (McCollister et al., 1951). The amount of $[^{14}C]$ carbon dioxide exhaled during the 7-day period following exposure was reported to be 10-20% of the total radioactivity expired. The authors integrated the resulting equation from 18 to 1,800 hours (75 days) and estimated that 4.4 mg or 11% of the total amount of radioactivity eliminated was excreted as carbon dioxide.

Shah et al. (1979) studied the metabolism of $[^{14}C]$ carbon tetrachloride by rat liver in vitro. Samples of liver homogenate equivalent to 0.167 g of tissue were incubated for 30 minutes at 37.5°C with 10 umole of ^{14}C-labeled carbon tetrachloride alone, and with either NADH or NADPH or both. $[^{14}C]$ carbon dioxide was detected by scintillation counting. The results are shown in Table III-4. The addition of NADPH appeared to result in substantial conversion of carbon tetrachloride to carbon dioxide. Addition of NADH and NADPH did not increase the conversion over that seen with NADPH alone.

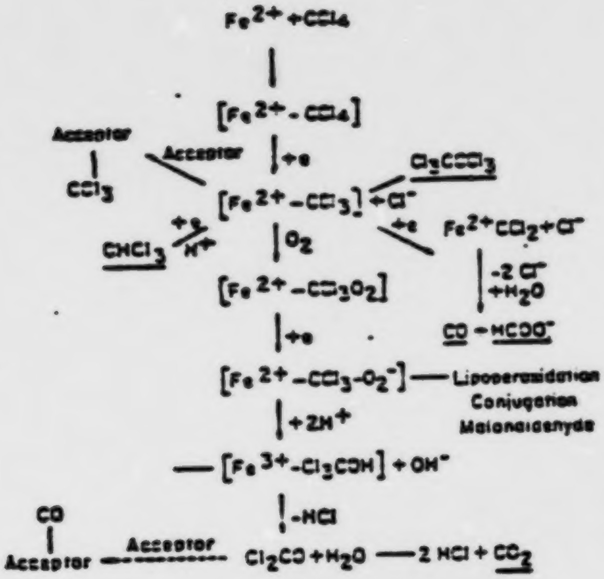
Shah et al. (1979) tested for the possible formation of carbonyl chloride in hepatic carbon tetachloride metabolism by adding L-cysteine to the in vitro rat liver system described above. Carbonyl chloride and L-cysteine are known to react chemically to form a condensation product, 2-oxothiazolidine-4-carboxylic acid. The presence of the condensation product was confirmed by thin-layer chromatography and mass spectrometry. The author inferred from the presence of 2-oxothiazolidine-4-carboxylic acid that carbonyl chloride was formed in the metabolism of carbon tetrachloride by rat liver microsomes. The authors postulated a mechanism of biotransformation for carbon tetrachloride which involves a sequential oxidation of carbon tetrachloride while bound to a heme (see Figure III-1). Release of bound intermediates then gives rise to different unrelated metabolites at the site of release.

TABLE III-4 Conversion of $[^{14}C]$ Carbon Tetrachloride to $[^{14}C]$ Carbon Dioxide by Rat Liver Homogenate

Nucleotide added	$[^{14}C]CO_2$ (mole/g liver, mean ± SEM)
None	27 ± 5
NADH	373 ± 17
NADPH	464 ± 33
NADH + NADPH	472 ± 21

Source: Shah *et al.* (1979)

Fowler (1969) detected hexachloroethane and chloroform in the tissues of rabbits orally administered carbon tetrachloride. A total of 15 rabbits were given carbon tetrachloride



$Fe^{2+} + CCl_4$
$[Fe^{2+}-CCl_4]$
Acceptor
CCl_3
Acceptor
+e
$\underline{Cl_3CCCl_3}$
$[Fe^{2+}-CCl_3] + Cl^-$
+e
$\underline{CHCl_3}$
H^+
O_2
+e
$Fe^{2+}CCl_2 + Cl^-$
$-2\,Cl^-$
$+H_2O$
$[Fe^{2+}-CCl_3O_2]$
+e
$\underline{CO} + HCOO^-$
$[Fe^{2+}-CCl_3-O_2^-]$
Lipoperoxidation
Conjugation
Malonaldehyde
$+2H^+$
$[Fe^{3+}-Cl_3COH] + OH^-$
-HCl
CO
Acceptor
Acceptor
$Cl_2CO + H_2O$
$2\,HCl + \underline{CO_2}$

Figure III-1 Pathways of Carbon Tetrachloride Metabolism. Products identified as carbon tetrachloride metabolites are underlined. The electrons utilized in the reactions are assumed to come from NADH or NADPH via the flavoprotein cytochrome reductases. Fe^{2+} and Fe^{3+} denote the respective ferro- and ferricytochromes. Redrawn from Shah et al. (1979).

at 1 ml/kg bw and sacrificed in groups of five at 6, 24, and 48 hours after exposure. Samples of fat, liver, kidney, and muscle tissues were analyzed for chloroform and hexachloroethane by gas chromatography. The results of the analysis are in Table III-5. The fat contained the highest amounts of hexachloroethane at each sampling time, but the highest concentrations of chloroform appeared in the liver.

Excretion

McCollister et al. (1951) studied the elimination of ^{14}C-labeled carbon tetrachloride from rhesus monkeys exposed by

TABLE III-5 Chloroform and Hexachloroethane in Tissues of Rats Given Carbon Tetrachloride Orally

Sample time	Tissue	$CHCl_3$ (ug/g tissues)	Cl_3CCCl_3 (ug/g tissues)
6 hours	Fat	4.7 ± 0.5	4.1 ± 1.2
	Liver	4.9 ± 1.5	1.6 ± 0.5
	Kidney	1.4 ± 0.6	0.7 ± 0.2
	Muscle	0.1 ± 0.1	0.3 ± 0.2
24 hours	Fat	1.0 ± 0.2	16.5 ± 1.5
	Liver	1.0 ± 0.4	4.2 ± 1.8
	Kidney	0.4 ± 0.2	2.2 ± 1.1
	Muscle	0.1 ± 0.1	0.5 ± 0.2
48 hours	Fat	0.4 ± 0.1	6.8 ± 2.4
	Liver	0.8 ± 0.2	1.0 ± 0.3
	Kidney	0.2 ± 0	Trace
	Muscle	0.1 ± 0.1	Trace

Source: Fowler (1969)

inhalation for 344 minutes. The total ^{14}C radioactivity in the blood decreased 12% during the first 10 minutes after exposure. Graphs of data from the analysis of blood samples obtained periodically for 10-12 days following exposure showed that the level of carbon tetrachloride in the blood decreased exponentially with time. At 10 days, the level of carbon tetrachloride in blood was approximately 0.009 mg/100 g. The authors estimated that 21% of the total amount of carbon tetrachloride absorbed was eliminated in expired air during the first 18 days. By extrapolation of these data, the authors concluded that over 1,800 hours (75 days) approximately 51% of the carbon tetrachloride initially absorbed would be eliminated in exhaled breath either as carbon tetrachloride or carbon dioxide. Analysis of urine and feces showed measurable amounts of radioactivity after 15 and 12 days, respectively. The authors interpreted these findings as indicating that significant quantities of carbon tetrachloride and/or metabolites may be excreted by these routes (breath, urine, and feces).

Summary

Carbon tetachloride is readily absorbed from the lungs and the gastrointestinal tract, as expected from its partition coefficients. Although few quantitative data are available on the amount of carbon tetrachloride absorbed through the lungs, the chemical and its metabolites have been reported in blood, many tissues, exhaled air, urine, and feces after administration by inhalation. Carbon tetrachloride has also been absorbed through the skin, but the reported rate of absorption was much slower than that of inhalation.

In published studies, carbon tetrachloride has appeared to be distributed to all major organs following absorption. The highest concentrations have been found in the liver, fat, blood, brain, kidney, spleen, and pancreas.

Carbon tetrachloride metabolism has been reported to occur primarily in the liver. Carbon tetrachloride has been postulated to be metabolized to a trichloromethyl radical bound to an iron atom in the cytochrome heme moiety. This trichloromethyl radical was reported to be either further metabolized or released as a free radical. The trichloromethyl free radical was reported to undergo a variety of reactions; including hydrogen abstraction to form chloroform, and dimerization to form hexachloroethane. Further metabolism of the heme-bound trichloromethyl radical was postulated to result in the eventual formation of carbonyl chloride (phosgene).

Carbon tetrachloride and its metabolites have been reported in many studies to be excreted primarily in exhaled air, and also in the urine and feces.

IV. HUMAN EXPOSURE

Humans may be exposed to carbon tetrachloride in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to carbon tetrachloride in the environment is presented in another document entitled "Occurrence of Carbon Tetrachloride in Drinking Water, Food, and Air" (Letkiewicz et al. 1983). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to carbon tetrachloride from sources other than the three considered here, notably in occupational settings and from the use of consumer products containing carbon tetrachloride. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various carbon tetrachloride concentrations. The 70-kg adult male is used for estimating dose, which takes into account the amount of the medium contacted (i.e., water and food ingested; air breathed) and the amount of carbon tetrachloride actually absorbed into the body.

a. Water

Cumulative estimates of the U.S. populations exposed to various carbon tetrachloride levels in drinking water from public drinking water systems are presented in Table IV-I. The values in the table were obtained using Federal Reporting Data Systems data (FRDS 1983) on populations served by primary water supply systems and the estimated number of these water systems that contain a given level of carbon tetrachloride. An estimated 26,810,000 individuals (12.5% of the population of 214,419,000 using public water supplies) are exposed to levels of carbon tetrachloride in drinking water at or above 0.5 ug/l, while 2,087,000 individuals (1.0%) are exposed to levels above 5 ug/l. It is estimated that 655,000 individuals are exposed to levels greater than 20 ug/l; none are estimated to be exposed to levels exceeding 30 ug/l.

Table IV-I. Total Estimated Cumulative Population (in Thousands) Exposed to Carbon Tetrachloride in Drinking Water Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of				
		≥ 0.5	>5	>10	>20	>30
Groundwater	73,473	2,157	121	43	0	0
Surface water	140,946	24,653	1,966	655	655	0
Total	214,419	26,810	2,087	698	655	0
(% of total)	(100%)	(12.5%)	(1.0%)	(0.3%)	(0.3%)	(0%)

No data were obtained on regional variations in the concentration of carbon tetrachloride in drinking water. The highest concentrations are expected to occur near sites of production and use of carbon tetrachloride and also, in the case of groundwater, near waste disposal sites.

Little information was available on gastrointestinal absorption rates for carbon tetrachloride. Because of its lipophilic nature, ingested carbon tetrachloride is expected to be readily absorbed. In one study, rats orally exposed to carbon tetrachloride were found to excrete at least 80% of the administered dose within 10 hours via the lungs, indicating that at least 80% of the dose was absorbed (Marchland et al. 1970 cited in USEPA 1982). Another study gave an absorption factor of 50% for ingestion, but the report has been criticized for not including substantiating information or citing the specific literature (Stokinger and Woodward 1958 cited in USEPA 1982). A conservative estimate for the rate of gastrointestinal absorption based on the limited data available is 100% (USEPA 1982).

Daily intake levels of carbon tetrachloride from drinking water were estimated using various exposure levels and the assumptions presented in Table IV-II. The data in the table suggest that the majority of the persons using public drinking water supplies would be exposed to intake levels below 0.014 ug/kg/day.

Table IV-II. Estimated Drinking Water Intake of Carbon Tetrachloride

Exposure level (ug/l)	Persons using supplies exposed to indicated levels		Intake (ug/kg/day)
	Population	% of Total population	
≥0.5	26,810,000	12.5%	≥0.014
>5.0	2,087,000	1.0%	>0.14
>10	698,000	0.3%	>0.29
>20	655,000	0.3%	>0.57

Assumptions: 70-kg man, 2 liters of water/day, gastrointestinal absorption rate of 100% (USEPA 1982).

An indication of the overall exposure of the total population to carbon tetrachloride can be obtained through the calculation of population-concentration values. These values are a summation of the individual levels of carbon tetrachloride to which each member of the population is exposed. An explanation of the derivation of these values is presented in Appendix C. Population-concentration estimates for carbon tetrachloride in drinking water were 3.3×10^7 ug/l x persons (best case), 9.7×10^7 ug/l x persons (mean best case), 1.1×10^8 ug/l x persons (mean worst case), and 2.5×10^8 ug/l x persons (worst case).

Assuming a consumption rate of 2 liters of water/day and a gastrointestinal absorption rate of 100%, population-dose values of 6.6×10^7 ug/day x persons (best case), 1.9×10^8 ug/day x persons (mean best case), 2.2×10^8 ug/day x persons (mean worst case), and 5.0×10^8 ug/day x persons (worst case) were derived.

b. Diet

The dietary intake of carbon tetrachloride in the United States was estimated using data on concentrations of carbon tetrachloride in food composites in the TEAM study (Pellizzari et al. 1982) and data on the average intake of food by food class (FDA 1980) (Table IV-III). Since levels of carbon tetrachloride in the composites for the TEAM survey were below the quantitation limit, daily intakes were calculated by assuming that these levels were equal to zero (minimum estimate) or to the quantitation limit (maximum estimate). Dietary intakes of carbon tetrachloride for the food classes studied, estimated by this method, varied between 0-1.27 ug/day.

Table IV-III. Estimated Adult Dietary Intake of Carbon Tetrachloride by Food Class[a] Using TEAM Survey Data

Food class		Average intake of food class (kg/day)[b]	Average level of carbon tetrachloride (ug/kg)		Average intake of carbon tetrachloride (ug/day)	
			Minimum[c]	Maximum[d]	Minimum	Maximum
I.	Dairy	0.753	0	1.0	0	0.75
II.	Meat, fish, and poultry	0.262	0	0.9	0	0.24
X.	Oils and fats	0.073	0	3.0	0	0.22
XII.	Beverages	0.128[e]	0	0.5	0	0.064
TOTAL					0	1.27

[a] Eight food classes not analyzed: grains and cereals (III), potatoes (IV), leafy legume and root vegetables (V, VI, VII), garden fruits (VIII), fruits (IX), and sugars and adjuncts (XI).

[b] From FDA 1980.

[c] All nonquantifiable values assumed to be equal to zero.

[d] All nonquantifiable values assumed to be equal to the quantitation limit (value reported is the average of those composites with known quantitation limits).

[e] Calculated by subtracting 14-day drinking water consumption from 14-day beverage consumption (FDA 1980) and dividing by 14.

Several problems arose in the use of the TEAM data:

1) The data are limited, since only five samples from each food group were analyzed, and they may not be representative of normal carbon tetrachloride levels in foods.

2) Only four of twelve food classes, those suspected of containing the highest levels of volatile organics, were analyzed.

3) Composite samples generally contained lower levels of carbon tetrachloride than expected from levels in the spiked subcomposite samples from which they were obtained (i.e., some carbon tetrachloride appeared to be lost during compositing).

4) The grains and cereals class, which may contain significant carbon tetrachloride as a result of grain fumigation, was not analyzed.

Because of these data limitations, the dietary intake values presented above are considered to be approximations.

Gastrointestinal absorption of carbon tetrachloride was discussed in the previous section. The estimated absorption rate was 100%. If the average adult male weighs 70 kg and has a daily intake of 1.27 ug of carbon tetrachloride (the maximum estimated in the TEAM study), the estimated adult dietary intake is 0.018 ug/kg/day.

It is expected that dietary levels of carbon tetrachloride vary somewhat with geographical location, with higher levels occurring in foods from areas near sources of carbon tetrachloride exposure. However, no estimates of variations in intake by geographical region could be made from the available data. Variances in individual exposure due to differences in diet also could not be assessed.

c. Air

Exposure to carbon tetrachloride in the atmosphere varies from one location to another. The highest level of carbon tetrachloride reported in the atmosphere was 69,000 ng/m^3 (69 ug/m^3) (Battelle 1977 cited in Brodzinsky and Singh 1982). High levels, averaging greater than 10,000 ng/m^3 (10 ug/m^3), have been detected in other areas. Normal levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated median air levels of carbon tetrachloride for rural/remote areas, urban/suburban areas, and source dominated areas of 820 ng/m^3 (0.82 ug/m^3), 1,200 ng/m^3 (1.2 ug/m^3), and 3,700 ng/m^3 (3.7 ug/m^3), respectively.

The monitoring data available are not sufficient to determine regional variations in exposure levels for carbon tetrachloride. However, urban and industrial areas appear to contain higher levels, as expected.

Pulmonary absorption rates for carbon tetrachloride have been reported in several studies. In one, rhesus monkeys inhaled ^{14}C-labeled carbon tetrachloride vapor at an average concentration of 290 mg/m^3, and absorption was measured as the difference between the concentrations of carbon tetrachloride in inhaled and exhaled air. An average absorption rate of 30.4% was obtained (McCollister et al. 1951 cited in USEPA 1982). In another study, the absorption of carbon tetrachloride by humans was studied by the difference in quantity of carbon tetrachloride in inhaled and exhaled air. The reported absorption range was 57-65% (Lehmann and Schmidt-Kehl 1936 cited in USEPA 1982). A further study reported an absorption factor for inhalation of 30% (Stokinger and Woodward 1958 cited in USEPA 1982). The last study, however, has been criticized for not including substantiating information or citing literature. From these data, a pulmonary absorption rate of 40% was estimated (USEPA 1982).

The daily respiratory intake of carbon tetrachloride from air was estimated using the assumptions presented in Table IV-IV and the median and maximum levels for carbon tetrachloride reported above. The estimates in Table IV-IV indicate that the daily carbon tetrachloride intake from air for adults in source dominated areas is approximately 0.5 ug/kg/day. In contrast, the intake calculated using the maximum carbon tetrachloride level reported is 9.1 ug/kg/day; few if any persons are believed to be exposed at that level. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Table IV-IV. Estimated Respiratory Intake of Carbon Tetrachloride

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (0.82)	0.11
Urban/suburban (1.2)	0.16
Source dominated (3.7)	0.49
Maximum (69)	9.1

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975), pulmonary absorption rate of 40% (USEPA 1982).

In addition to the available monitoring data, Systems Applications (1982) has provided estimates of atmospheric levels of carbon tetrachloride by applying air dispersion models to carbon tetrachloride emission sources. The computed average concentrations of carbon tetrachloride and the number of individuals estimated to be exposed to these concentrations are presented in Table IV-V. Specific point sources are individually identified sources with known locations and modes and rates of emissions. These are generally manufacturing plants. General point sources are those which are numerous, small, or of uncertain location. However, these sources can produce isolated patterns of significant concentration. Area sources are numerous and emit only small concentrations of the chemical (e.g., home chimneys, automobiles). These estimates indicate that less than 600,000 persons are exposed to airborne carbon tetrachloride at concentrations greater than 2,500 ng/m^3 (2.5 ug/m^3).

Table IV-V also presents a total population-concentration estimate for carbon tetrachloride of 6.45×10^7 ug/m^3 x persons. Assuming an inhalation rate of 23 m^3 of air/day and a 40% pulmonary absorption rate, a population-dose of 5.93×10^8 ug/day x persons was calculated.

SUMMARY

Table IV-VI presents a general view of the total amount of carbon tetrachloride received by an adult male from air, food, and drinking water. Four separate exposure levels in air, five exposure levels in drinking water, and one exposure level from foods are shown in the table.

The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to carbon tetrachloride at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

The relative source contribution data for carbon tetrachloride account for differential absorption rates for the chemical by the respiratory and gastrointestinal routes. Thus, relative doses of the chemical directly enter-

Table IV-V. Exposure and Dosage Summary for Airborne Carbon Tetrachloride

Concentration level (ug/m^3)	Population exposed (persons)				Dosage (ug/m^3 x persons)			
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	Area source	U.S. total
829	15	0	0	15	12,600	0	0	12,600
500	133	0	0	133	88,200	0	0	88,200
250	625	0	0	625	257,000	0	0	257,000
100	1,652	0	0	1,652	404,000	0	0	404,000
50	4,318	0	0	4,318	576,000	0	0	576,000
25	9,146	0	0	9,146	729,000	0	0	729,000
10	23,759	0	0	23,759	950,000	0	0	950,000
5	36,719	0	0	36,719	1,040,000	0	0	1,040,000
2.5	62,189	0	505,140	567,329	1,130,000	0	2,327,400	3,457,400
1	121,852	0	9,149,730	9,271,582	1,230,000	0	17,913,784	19,143,784
0.5	213,423	0	33,072,205	33,285,628	1,290,000	0	35,194,859	--
0.25	--	0	83,219,704	--	--	0	51,528,284	--
0.1	--	0	142,928,535	--	--	0	61,879,083	--
3.8×10^{-5}	7,979,115	0	158,679,135	--	1,530,000	0	62,926,300	64,456,300

Note: The use of "--" as an entry indicates that the incremental increase in the dosage or the population exposed is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

ing the body are compared. However, it should be noted that the relative effects of the chemical on the body may vary by different routes of exposure.

Brodzinsky and Singh (1982) calculated a median urban/suburban air level of carbon tetrachloride of 1.2 ug/m^3 based on air monitoring data. Assuming an air level of 1.2 ug/m^3 and the estimated carbon tetrachloride intake of 0.018 ug/kg/day in foods, drinking water would be the predominant source of carbon tetrachloride exposure in the adult male at drinking water levels above 6.3 ug/l. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of carbon tetrachloride in drinking water and low concentrations of carbon tetrachloride in ambient air and food are unknown.

Population-dose estimates for carbon tetrachloride in drinking water and air were presented previously. Estimates for drinking water ranged from 0.66-5.0 x 10^8 ug/day x persons; the estimate for ambient air was 5.9 x 10^9 ug/day x persons. These estimates suggest that ambient air may be a greater source of exposure to carbon tetrachloride than drinking water on a general population basis. Comparison of these estimates, however, may be deceiving since the same population-dose level can occur if: 1) a whole population is exposed to moderate levels of a chemical or 2) some segments of the same population are exposed to high levels and others to low levels. The population-dose values presented give no indication of the relative predominance of drinking water and air as specific sources of carbon tetrachloride on a site-by-site or subpopulation basis.

Table IV-VI. Estimated Dose of Carbon Tetrachloride Absorbed from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote ($0.82\ ug/m^3$)	Urban/suburban ($1.2\ ug/m^3$)	Source dominated ($3.7\ ug/m^3$)	Maximum ($69\ ug/m^3$)
0	0.13 (0%)	0.18 (0%)	0.51 (0%)	9.1 (0%)
0.5[a]	0.14 (9.9%)	0.19 (7.3%)	0.52 (2.7%)	9.1 (0.2%)
5.0[b]	0.28 (50%)	0.32 (44%)	0.65 (22%)	9.3 (1.5%)
10[c]	0.42 (69%)	0.47 (62%)	0.80 (36%)	9.4 (3.1%)
20[d]	0.71 (80%)	0.75 (76%)	1.1 (52%)	9.7 (5.9%)

Intake from each source (see Sections 5.1-5.3):

Water:	0.5 ug/l:	0.014 ug/kg/day
	5.0 ug/l:	0.14 ug/kg/day
	10 ug/l:	0.29 ug/kg/day
	20 ug/l:	0.57 ug/kg/day
Air:	$0.82\ ug/m^3$:	0.11 ug/kg/day
	$1.2\ ug/m^3$:	0.16 ug/kg/day
	$3.7\ ug/m^3$:	0.49 ug/kg/day
	$69\ ug/m^3$:	9.1 ug/kg/day
Food:		0.018 ug/kg/day

[a] 26,810,000 individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l (12.5% of population using public water supplies).

[b] 2,087,000 individuals using public drinking water systems are estimated to be exposed to levels > 5.0 ug/l (1.0% of population using public water supplies).

[c] 698,000 individuals using public drinking water systems are estimated to be exposed to levels > 10 ug/l (0.3% of population using public water supplies).

[d] 655,000 individuals using public drinking water systems are estimated to be exposed to levels > 20 ug/l (0.3% of population using public water supplies).

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V. HEALTH EFFECTS IN ANIMALS

This section discusses the acute and chronic effects of carbon tetrachloride exposure, with emphasis on studies in which dose-response relationships for minimal adverse effects have been developed. The subsection on teratogenicity, mutagenicity, and carinogenicity outline the various study designs in more detail and elaborate on their deficiencies where appropriate.

Acute Effects

The acute toxicity of carbon tetrachloride has been extensively documented. This subsection will concentrate on those studies that (i) describe nonlethal effects and (ii) provide data on a range of doses from which dose-response relationships can be determined. For this reason a number of studies referring to LD_{50}'s will not be discussed. However, Table V-1 summarizes some of the lethal dose data reported for carbon tetrachloride in various species.

Liver Effects. Functional changes in mouse liver as a result of carbon tetrachloride exposure were measured by increases in the activity of the enzyme serum glutamic-pyruvic transaminase (SGPT) and in bromsulfophthalein (BSP) retention (Klaassen and Plaa, 1966). Male Swiss-Webster mice were administered various amounts of analytical grade carbon tetrachloride intraperitoneally in corn oil at a final volume of 10 ml/kg

TABLE V-1 Toxic Doses and Effects of Carbon Tetrachloride in Animals

Animal	Route of administration	Effect[a]	Dose
Rat	Oral	LD_{50}	2,800 mg/kg
Mouse		LD_{50}	12,800 mg/kg
Dog		LD_{LO}	1,000 mg/kg
Rabbit		LD_{50}	6,380 mg/kg
Rat	Intraperitoneal	LD_{50}	1,500 mg/kg
Mouse		LD_{50}	4,675 mg/kg
Dog		LD_{LO}	1,500 mg/kg
Rabbit		LD_{LO}	478 mg/kg
Rat	Inhalation	LC_{50}	4,000 ppm/4 hrs
Mouse		LC_{50}	9,526 ppm/8 hrs
Cat		LC_{LO}	38,110 ppm/2 hrs
Guinea pig		LC_{LO}	20,000 ppm/2 hrs
Cat	Subcutaneous	LD_{LO}	300 mg/kg
Rabbit		LD_{LO}	3,000 mg/kg

[a] LD_{50}, dose lethal for 50% of animals

LC_{50}, concentration lethal for 50% of animals

LD_{LO}, lowest lethal dose

Source: NIOSH (1978)

of body weight (bw). Mice treated only with corn oil were used to establish the normal range of values for BSP retention and SGPT activity, which were determined 24 hours after treatment. The authors reported the median effective doses of carbon tetrachloride as 15.9 mg/kg bw for elevation of SGPT activity and 94 mg/kg bw for BSP retention. The authors did not specify the range of carbon tetrachloride doses used or the number of animals used at each dose.

In another study, Klaassen and Plaa (1967) further defined a dose-response relationship for carbon tetrachloride exposure and elevated SGPT levels in mice. They used the "up and down" method in which one dose of the compound was given to an animal and the animal's SGPT activity 24 hours after the dose was noted. If the enzyme was elevated, the dose was decreased 40% and the experiment repeated in another animal. If no effect was noted, the dose was increased 40% and the experiment repeated in another animal. This series was repeated three times after one positive and one negative response had been obtained. The results for mice are shown in Table V-2. The authors concluded that 13 mg/kg bw was the median effective dose of carbon tetrachloride in mice as measured by elevated SGPT values.

Table V-2 SGPT Values of Mice Administered Carbon Tetrachloride Intraperitoneally in "Up and Down" Experiment

Animal	Dose (mg/kg bw)	Response[a]
1	17.5	E
2	13	N
3	17.5	E
4	13	E
5	8	N
ED_{50}	13	

[a]E = Elevated SGPT after 24 hours

N = Normal SGPT after 24 hours

Source: Klaassen and Plaa (1967)

Sein and Chu (1979) studied the effect of carbon tetrachloride on the level of the liver enzyme glucose-6-phosphatase in mice. Groups of six male LAC strain mice were treated intraperitoneally with 795, 1,590, or 3,180 mg/kg bw of carbon tetrachloride (purity unspecified) in paraffin oil. The animals were sacrificed 24 hours after treatment. Control animals, number unspecified, were given paraffin oil and sacrificed on the same schedule. The livers were removed and analyzed for glucose-6-phosphatase. The results of the analysis showed that after treatment with carbon tetrachloride at 795 or 1,590 mg/kg, the enzyme level fell to 40% of the control value. At a dose of 3,180 mg/kg, the enzyme level had decreased to 20% of the control value.

A series of experiments to determine the effects of single carbon tetrachloride exposures on rats were performed by Murphy and Malley (1969). Adult male Holtzman rats (250-350 g) were orally administred various doses of undiluted carbon tetrachloride by gavage. Control animals were administered equal volumes of water. At 2-20 hours after treatment, animals were sacrificed and liver enzyme activities and liver weights were measured. The results are shown in Table V-3. The animals receiving carbon tetrachloride at 1,600 mg/kg bw were sacrificed 20 hours after treatment and the livers examined histopathologically. The examination showed extensive fatty infiltration, inflammation, and some centrolobular necrosis. The liver-to-body weight ratios were also increased.

Murphy and Malley (1969) also determined the effect of single exposures to carbon tetrachloride on the activities of the corticosterone-inducible liver enzymes tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase. Groups of rats (4-6 in each group, 8 untreated controls) were treated with carbon tetrachloride (0, 400, 800, and 1,600 mg/kg bw) and sacrificed 5 hours after treatment. Data showed that the enzyme levels were increased roughly in proportion to the dose.

Similar studies on the effect of carbon tetrachloride administration on serum activity of liver enzymes in rats were performed by Drotman and Lawhorn (1978). Groups of four male Cox rats were admministered carbon tetrachloride intraperitoneally at 60, 120, 240, or 480 mg/kg bw in a total volume of 1 ml in corn oil and exsanguinated at specified time intervals. Serum activities were determined for the enzymes sorbitol dehydrogenase (SSDH), ornithine carbamyl transferase (SOCT), aspartate aminotransferase (SAST), and isocitric dehydrogenase (SICDH). Liver specimens were taken from each animal and scored for histopathologial changes. The results of the enzyme analyses and histopathology are tabulated in Table V-4 by dose and hours after dose. The SOCT activities showed the best correlation with liver histopathology in time of appearance as well as extent of damage. The authors concluded that SOCT levels are a sensitive indicator of liver damage.

Effects of acute exposure to low levels of carbon tetrachloride were also reported by Korsrud _et al_. (1972). Male Wistar rats (260-400 g; 8-10 animals per treatment group) were administered single oral doses of carbon tetrachloride

Table V-3 Effects of Oral Carbon Tetrachloride on Liver Weight and Liver and Plasma Enzyme Activities in Male Rats

Dose (mg. kg bw)	Time (hr)	Number of animals	Plasma AKT[a]	Liver[b]			
				AKT[a]	TKT[a]	AP[a]	Weight (g/100g bw)
0	-	7	2.6 ± 0.3[b]	2360 ± 182	93 ± 11	14.9 ± 1.0	2.75 ± 0.06
3200	2	4	2.1 ± 0.2	-	170 ± 12	14.5 ± 1.0	2.94 ± 0.04
3200	5	5	13.2 ± 3.4	1813 ± 331	330 ± 36	14.2 ± 1.3	3.26 ± 0.10
3200	20	5	35.2 ± 4.2	1174 ± 559	361 ± 42	34.6 ± 5.8	4.36 ± 0.06
2400	20	4	35.2 ± 3.4	1585 ± 148	305 ± 21	37.1 ± 1.6	3.95 ± 0.07
1600	20	5	18.1 ± 4.3	1596 ± 194	294 ± 61	33.3 ± 3.6	3.90 ± 0.05
800	20	4	9.3 ± 2.3	2120 ± 182	138 ± 5	29.1 ± 6.5	3.47 ± 0.07

[a] AKT = alanine-α-ketoglutarate transaminase

TKT = tyrosine-α-ketoglutarate transaminase

AP = alkaline phosphatase

b = means ± SE in micromoles of product formed per gram of fresh liver or milliliter of plasma per hour

Adapted from Murphy and Malley (1969)

(0-4,000 mg/kg bw) in corn oil (5 ml/kg bw). The rats were fasted for 6 hours before treatment and for 18 hours afterward, and then sacrificed. Assays included liver weight and fat content, serum urea and arginine levels, and levels of nine serum enzymes produced mainly in the liver. At 20 mg/kg bw there was histopathologic evidence of toxic effects on the liver. These changes included a loss of basophilic stippling, a few swollen cells, and minimal cytoplasmic vacuolation. At 40 mg/kg bw, liver fat, liver weight, serum urea, and levels of five of the nine liver enzymes were increased while serum arginine decreased. At higher doses the remaining four enzyme levels were also elevated.

Alumot et al. (1976) reported the effects of subchronic exposure to feed that had been fumigated with carbon tetrachloride. Groups of six weaning rats 4 weeks old were fed a diet containing carbon tetrachloride at 150, 275, or 520 mg/kg of feed for 5 weeks (females) or 6 weeks (males). The fumigated feed was stored in airtight containers; carbon tetrachloride loss during the storage period of 7-10 days was determined to be 5%. The animals were allowed access to the feed only at set time intervals to minimize loss of carbon tetrachloride by volatilization. The authors calculated that the amount of carbon tetrachloride remaining in the consumed feed was 60-70% of the amount initially present;

Table V-4 Effects of Carbon Tetrachloride on Liver Histopathology and Serum Enzyme Levels

Dose (mg/kg)	Hours after dose	Histology[a]	Serum enzyme concentrations relative to pretreatment levels			
			SOCT	SSDH	SAST	SICDH
60	0	0	1.0	1.0	1.0	1.0
	6	2	9.6*	2.5	2.0	1.4
	12	1	8.2*	4.4*	2.5*	1.1
	24	1	5.7*	1.7	2.0	1.3
	36	0	1.0	1.0	1.0	1.0
120	0	0	1.0	1.0	1.0	1.0
	24	3	14.0*	7.2*	2.1*	1.1
	48	2	7.4*	1.0	1.0	1.0
	96	1	1.8	1.7	1.3	1.3
	168	0	1.0	1.0	1.0	1.0
240	0	0	1.0	1.0	1.0	1.0
	24	3	31.0*	17.4*	5.8*	7.2*
	48	4	180.0*	43.4*	17.0*	7.4
	96	1	6.6*	4.7*	3.6*	2.0
	168	0	1.1	1.0	1.9	2.0
480	0	0	1.0	1.0	1.0	1.0
	24	3	28.4*	90.0*	6.1*	5.4*
	48	4	465.5*	163.5*	18.4*	50.4*
	96	1	1.0	8.4*	1.8*	2.0
	168	0	1.0	1.4	1.0	2.1

[a] 0 = No observable changes.

1 = Minimal changes. Large central vein, swelling of hepatocyte, etc.

2 = Mild degenerative change. Loss of cord arrangement.

3 = Moderate degenerative change. Pale cytoplasm, spindle cell.

4 = Marked degenerative change. Centrilobular fatty degeneration.

* Significantly different from zero time as determined by one-way analysis of variance of the log-transformed data ($P \leq .01$).

Adapted from Drotman and Lawhorn (1978)

the total decrease reflected amounts lost during storage and after removal from storage to feeding troughs. From these data and the weights of the animals, the authors calculated that 275 mg/kg of feed represented a daily dose of 40 mg/kg bw. (By assuming that all parameters were the same and that the delivered dose was proportional to the concentration in feed, diets of 150 and 520 mg/kg of feed were calculated by JRB to represent daily doses of 22 and 76 mg/kg bw, respectively.) At the end of the experiment the animals were weighed and sacrificed. Of the three doses, only the highest, 76 mg/kg bw (520 mg/kg of feed), caused significantly depressed weight gain in males. Weight gain in females appeared to be unaffected by all doses. Total lipid and triglyceride levels in the liver were significantly higher in animals fed carbon tetrachloride at 40 and 76 mg/kg bw than in controls or animals fed 22 mg/kg bw. Levels of liver phospholipids (measured in females) were not affected at any dose. Of the three doses used in this experiment, the lowest, 22 mg/kg bw, failed to produce effects on the measured parameters.

In addition to the study of hepatic effects of carbon tetrachloride in mice, described earlier in this section, Klaassen and Plaa (1967) also investigated the hepatic effects of carbon tetrachloride exposure in dogs. Male and female mongrel dogs were treated intraperitoneally with carbon tetrachloride at 22-38 mg/kg bw in an "up and down" experimental design.

Blood samples were taken for measurement of SGPT 24 hours after administration of carbon tetrachloride. Control dogs had serum SGPT activity of 36±7 units. Therefore, 36±2 standard deviations or 50 units were chosen as the upper limit of the normal value. The results of the analysis are shown in Table V-5.

The SGPT values returned to normal in 17-18 days. Animals were then sacrificed and the livers were examined histopathologically. They showed moderate vacuolation of the centrolobular and midzonal hepatocytes as well as traces of brown material in the cytoplasm of centrolobular Kupffer cells.

Table V-5 SGPT Activity in Dogs 24 Hours After Intraperitoneal Administration of Carbon Tetrachloride in "Up and Down" Experiment

Animal	Dose (mg/kg)	Response[a]
1	22.2	N
2	30.2	E
3	22.2	N
4	30.2	N
5	38	E
ED_{50}	32	

[a] N = normal SGPT after 24 hours

E = elevated SGPT after 24 hours

Adapted from Klaassen and Plaa (1967)

Kidney Effects. Carbon tetrachloride, even at high doses, failed to induce renal failure as measured by phenolsulfonphtalein (PSP) excretion in mice although pathological kidney alterations were present (Plaa and Larson, 1965). Male Swiss mice (18-30 g) were given intraperitoneal injections of carbon tetrachloride (1,6006,400 mg/kg bw) dissolved in corn oil at a final volume of 0.1 ml/10 g bw. The animals were then hydrated with tap water (50 ml/kg bw) by gavage and placed on a urinary collection unit for 2 hours. Even carbon tetrachloride doses lethal in some animals (≥ 6,400 mg/kg bw) failed to cause renal dysfunction, measured as excretion of PSP, urinary protein, and glucose, in the majority of survivors. At a high nonlethal dose (3,260 mg/kg bw) minimal renal dysfunction was observed after 96 hours. Histologic examination of kidney sections from five mice that had been administered this dose showed necrosis of proximal convoluted tubules (n=1) and swelling of the tubules (n=4).

Carbon tetrachloride decreased the activity of glucose-6-phosphatase in the kidney (Sein and Chu, 1979). Male mice (40-50 days old, weighing 24-28 g) were injected intraperitoneally with carbon tetrachloride at 795, 1,590, or 3,180 mg/kg bw in paraffin oil. Twenty-four hours after injection of 795 or 1,590 mg/kg bw, the renal glucose-6-phosphatase activity decreased to 77% or 65% of the control value, respectively. Increasing the dose to 3,180 mg/kg bw had no further effect on the kidney enzyme level.

These results were in contrast to the liver glucose-6-phosphatase level discussed earlier, which decreased to 40% of the control value at the two lower doses and decreased further to 20% of the control value at 3,180 mg/kg bw. The authors attributed these differences to the limited metabolic capacity of the kidneys.

Klaassen and Plaa (1967) studied the effect of carbon tetrachloride exposure on kidney function in dogs. PSP excretion of less than 39% of control values was considered indicative of renal dysfunction. An unspecified number of male and female mongrel dogs were treated intraperitoneally with carbon tetrachloride at 22-38 mg/kg bw and the 24 hour excretion rate for PSP was determined. Control dogs were used to determine a normal range for PSP excretion. None of the dogs treated with carbon tetrachloride exhibited decreased PSP excretion. However, on histological examination of the kidneys from the treated dogs, the Bowman's capsules appeared dilated with some contraction of glomerular tufts and calcification of a small number of tubules in the medulla.

Lung Effects. Boyd et al. (1980) investigated the effect of ingestion and inhalation of carbon tetrachloride on pulmonary Clara cells in Swiss mice. For the ingestion study, the mice were treated with carbon tetrachloride (4,000 mg/kg bw) in a 50% sesame oil solution and sacrificed 16 hours after treatment. The lungs were removed and examined by electron microscopy. Clara cells exhibited massive dilation of vesicles of smooth endoplasmic reticulum, increased mitochondrial staining density, ribosomal disaggregation, nuclear condensation, and occasional cellular necrosis. Additional experiments with oral carbon tetrachloride doses of less than 1,600 mg/kg bw did not produce any pulmonary lesions visible by light microscopy. Doses of 2,400-4,800 mg/kg bw produced Clara cell lesions similar on electron microscopic examination to those previously described. The extent of damage was proportional to the dose administered. Boyd et al. (1980) also studied the time course of the Clara cell damage caused by ingestion of carbon tetrachloride (4,000 mg/kg bw). Pulmonary tissue was evaluated by light microscopy at 12, 24, 36, 48, 96, and 168 hours. The lesions were present at 12 hours, maximal at 24 hours, and less intense at 36 hours. By 48 hours, the lesions were seen infrequently and at 96 and 168 hours the pulmonary bronchioles appeared normal.

The pulmonary toxicity of inhaled carbon tetrachloride was also studied by Boyd et al. (1980). Swiss mice were exposed to carbon tetrachloride vapor at 71,800, 144,000, 287,000 or 574,000 mg/m^3 for 60, 60, 12, or 2 minutes, respectively. The animals were sacrificed 24 hours after exposure, and the lungs examined. Marked Clara cell lesions similar to those seen after oral exposure were seen at all exposure levels and necrosis was reported to be more frequent after inhalation than after oral exposure, but no effort to quantify this finding was reported.

Gould and Smuckler (1971) investigated the structural alterations in rat lungs following carbon tetrachloride ingestion. Male Sprague-Dawley rats (200-250 g) were fasted 16 hours prior to administration of carbon tetrachloride (4,000 mg/kg bw) by gavage. The animals exhibited piloerection and lassitude 3-4 hours after treatment. They were sacrificed 1, 4, 8, 12, or 24 hours following treatment. Necropsies were performed on all animals. Microscopic examination of the lungs of treated rats revealed perivascular edema and mononuclear infiltration in the first 4 hours after treatment. These areas were local but were estimated to involve 10% of the parenchyma. Areas of atelectasis and intraalveolar hemorrhage involving 15-20% of the parenchyma were observed 8-12 hours after treatment.

Electron micrographs of rat lungs after carbon tetrachloride ingestion showed granular pneumocytes containing swollen inclusions with decreased osmiophilia and attenuated lamellae 1 hour after treatment (Gould and Smuckler, 1971). These changes were more severe 4 hours following treatment. By 4-8 hours after treatment, cytoplasmic edema, dislocation of dense ribosomal aggregates, and mitochondrial disruption were apparent. Multivesicular bodies were "conspicuously decreased" within the granular pneumocytes. Necrosis was evident 12-24 hours after treatment. One hour after administration, endothelial cells displayed markedly increased pinocytotic vesicles. Severe disruption of endothelial cells was evident from 8 hours onward. Ultrastructural damage was seen in all components of the alveolar wall, and fibrin was observed within alveoli. The authors interpreted these findings as indicative of significant alterations in vascular permeability.

Lesions of the Clara cells in the lungs of male Sprague-Dawley rats orally treated with carbon tetrachloride were observed by Boyd _et al_. (1980). The carbon tetrachloride was administered by gavage at doses of 3,816, 5,088, and 7,155 mg/kg as a 50% solution in sesame oil. Control animals received sesame oil only. Clara cell lesions occurred at the two highest doses. The authors stated that the lesions were less pronounced than those in mice exposed to comparable amounts of carbon tetrachloride.

Chronic Effects

Smyth *et al*. (1936) studied the chronic effects of carbon tetrachloride inhalation exposure in rats. Groups of 24 Wistar rats were exposed to carbon tetrachloride concentrations of 315, 630, 1,260, or 2,520 mg/m^3 (50, 100, 200, or 400 ppm) for 8 hours a day, 5 days a week for 10.5 months. The carbon tetrachloride was found to contain less than 0.003% carbon disulfide. Control rats were used, but the number was unspecified. Growth retardation was observed at 2,520 mg/m^3. At 630 and 1,260 mg/m^3, growth was the same as in controls, and at 315 mg/m^3 growth was stimulated. Cirrhosis developed in rats exposed to 630, 1,260, and 2,520 mg/m^3 after 173, 115, and 54 exposures, respectively. When exposure was stopped, fatty liver degeneration resolved within 50 days. Surface alterations (hobnail liver) did not resolve until 156 days after cessation of exposure. Unspecified renal damage was observed after 52 exposures to 315 mg/m^3 and after 12-20 exposures at the higher concentrations.

In a chronic oral exposure study (Alumot *et al*., 1976), groups of 36 rats (18 male and 18 female littermates) were fed mash containing carbon tetrachloride at 0, 80, or 200 mg/kg of feed. The feed was stored in airtight containers, assayed for carbon tetrachloride content, and consumed soon after removal to feeding troughs. The authors calculated that 200 mg/kg of feed represented a daily dose of 10-18 mg/kg bw. After 2 years, the surviving animals were sacrificed. In these animals, serum values for glucose, protein, albumin, urea, uric acid, cholesterol, SGOT, and SGPT in the treated animals did not differ from those in controls. No fatty livers were detected in the treated animals. Thus, the authors found no biochemical abnormalities attributable to carbon tetrachloride exposure. However, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals. More than half the animals were dead at 21 months, although at 18 months the survival ranged from 61-89%. The authors indicated that 10-18 mg/kg bw (200 mg/kg of feed) is a no-adverse-effect level of carbon tetrachloride over 2 years. However, this conclusion may be questioned because of the poor survival rate and widespread respiratory infection of experimental animals.

Groups of 24 guinea pigs were exposed to carbon tetrachloride vapor at 315, 635, 1,260, 2,500 mg/m^3 in a study by Smyth et al. (1936). The frequency of exposure was 8 hours per day, 5 days per week for up to 10.5 months. Marked mortality occurred in exposed animals: 9/24 at 315 mg/m^3 after a median of 44 exposures (exposure terminated at 135 days), 16/24 at 630 mg/m^3 after a median of ten exposures, 13/24 at 1,260 mg/m^3 after a median of three exposures, and 19/24 at 2,520 mg/m^3 after a median of three exposures.

Guinea pigs exposed to the 315 mg/m^3 dose in the Smyth et al., (1936) study developed cirrhosis and hobnail surface alterations of the liver in 105 exposures. The authors concluded that survival of guinea pigs at higher doses was of insufficient duration to allow development of cirrhosis. In addition, granular swelling was observed in adrenal glands of guinea pigs exposed to carbon tetrachloride at 315, 630, and 260 mg/m^3 for 8, 7, and 17 exposures, respectively. Exposure to higher concentrations (1,260 or 2,520 mg/m^3) or continued exposure to lower concentrations resulted in marked damage to the sciatic nerves. Dense clumps of black granules (osmic acid stain) were observed paralleling the large majority of fibers.

Prendergast et al. (1967) repeatedly exposed 15 guinea pigs to carbon tetrachloride (purity unspecified) at 515 mg/m^3 (82 ppm) over a period of 6 weeks and observed hepatic changes. Three guinea pigs died on days 20, 22, and 30, respectively. All the animals showed a body weight loss. The surviving animals were sacrificed at 6 weeks and the livers examinined histopathologically. The examiniation revealed fatty infiltration, fibrosis, bile duct proliferation, hepatic cell degeneration and regeneration, focal inflammatory cell infiltration, alteration of lobular structure, and early portal cirrhosis. The hepatic lipid content of carbon tetrachloride-treated animals (35.4 ± 10.7%) was higher than that of the controls (11.0 ± 3.6%).

In addition, Prendergast et al. (1967) exposed continuously guinea pigs to carbon tetrachloride vapor at 61 mg/m^3 (10 ppm) for 90 days. Three of the 15 guinea pigs died: on days 47, 63, and 74 respectively. All the exposed animals showed a depressed weight gain. A "high incidence" of enlarged and discolored livers was reported on gross pathological examination. Histopathologic examination of the livers revealed fatty changes, fibroblastic proliferation, collagen deposition, hepatic cell degenertion and regeneration, and structure alteration of the liver lobule. Enzymatic studies showed that only the succinic dehydrogenase (SDH) activity was moderately reduced as compared to that in controls.

In addition to their studies on guinea pigs, Prendergast et al. (1967) studied the effects of both repeated and continuous exposure to carbon tetrachloride on three squirrel monkeys, three New Zealand rabbits, and two beagle dogs for each exposure regimen. The experimental designs were the same as those described for the guinea pigs. All the animals showed a weight loss during repeated exposure to 515 mg/m^3 (82 ppm). Fatty changes were noted in the liver of all species; they were most severe in rabbits, followed by dogs and monkeys. In the continuous exposure to 61 mg/m^3 (10 ppm) for 90 days, all species exhibited a depressed weight gain, as did guinea pigs. Liver changes were also noted, but enzyme activities (as measured by NADH, NADPH, SDH, LDH G6PI) were within the normal range. At a continuous exposure of 6.1 mg/m^3 (1 ppm), no toxic signs were noted.

Teratogenicity

In two studies on the teratogenic and prenatal toxicologic effects of carbon tetrachloride, the chemical was reported to produce prenatal toxicity following inhalation exposure.

Schwetz et al. (1974) exposed pregnant rats to carbon tetrachloride at 1,800 or 6,300 mg/m^3 (300 or 1,000 ppm) for 7 hours per day on days 6-15 of gestation. Statistically significant decreases in fetal body weight and crown-rump length were observed. Other parameters examined were not significantly different from those of controls. The authors concluded that carbon tetrachloride was not teratogenic at the concentrations used in this experiment. Two other statistically significant fetal effects were noted: an increased incidence of sternebral anomalies in the 6,300 mg/m^3 group and an increased incidence of subcutaneous edema in the 1,800 mg/m^3 group. Edema was not seen in the 6.300 mg/m^3 group. The dams exposed to both concentrations of carbon tetrachloride showed a statistically significant decrease in weight gain and also decreased food consumption compared to control animals. Hepatotoxicity as measured by significantly increased SGPT activity was also seen in the dams. However, the authors did not establish any consistent pattern between fetal toxicity and maternal toxicity at the subanesthetic levels of carbon tetrachloride used in this experiment.

Another study reported no teratogenic effects following exposure of pregnant rats to carbon tetrachloride at

1,575 mg/m^3 (250 ppm) 8 hours per day for 5 consecutive days between days 10-15 of pregnancy (Gilman, 1971). Concomitant exposure to 15% ethanol in drinking water also did not result in teratogenic effects. Carbon tetrachloride exposure, however, did decrease the viability index to 83% as compared to 99% for controls. The lactation index was also decreased to 83% as compared to 98% for controls. A decrease in the number of pups per litter also occurred: 9.2 as compared to 10.3 for controls. Concomitant ethanol exposure exacerbated this effect: 8.48 pups per litter as compared to 10.3 for controls.

No teratogenic effects were noted in rats fed diets containing carbon tetrachloride at either 80 or 200 mg/kg of feed for up to 2 years (Alumot et al., 1976). Groups of 18 female rats were mated at 3 months of age (6 weeks of exposure) with untreated males. Thereafter, they were mated every 2 months with groups of treated males.

Exposure to carbon tetrachloride in utero has been reported to result in liver damage in rat fetuses and neonates (Bhattacharyya, 1965). In one case, subcutaneous administration of carbon tetrachloride at 1,600 mg/kg bw to a pregnant rat on day 20 of gestation resulted in small areas of focal hepatic necrosis in a fetal liver 24 hours later. Similar treatment resulted in focal hepatic necrosis in neonates born 48 or 72 hours after treatment of dams on day 19 or 20 of gestation. Histologic findings generally included a sharply demarcated area of centrolobular necrosis and proliferative changes in nonnecrotic lobes.

In addition, fetuses were directly treated with carbon tetrachloride by subjecting the mother to a laparotomy and either injecting the chemical directly into the fetus or into the amniotic sac through the uterine wall (Bhattacharyya, 1965). Liver changes following injection of 6 mg of carbon tetrachloride were variable: cells generally became extremely pale in centrolobular and midzonal areas, indicating fatty infiltration. Livers remained abnormal until at least 4 days after birth. No necrosis, hemorrhage, or regeneration was observed.

Sensitivity of neonate rats to carbon tetrachloride was reported to be low 1 hour after birth, then to rise above the adult level at 19 hours and to decline by 3-7 days after birth. Thus, only two of ten 1-hour-old neonates receiving carbon tetrachloride (1,600 mg/kg) subcutaneously showed centrolobular necrosis after 24 hours. In addition, hepatic portal areas contained numerous neutrophils, but no bile duct proliferation could be observed, in contrast to findings in adult animals. Following the same treatment, 19-hour neonates showed more pronounced hepatic damage than 1-hour neonates. Damage declined in 3-and 4-day old neonates;

that in 5-,6-, and 7-day-olds was similar in appearance to that of adults.

Carbon tetrachloride can apparently be transferred to the neonates through mother's milk (Bhattacharyya, 1965). Subcutaneous administration of carbon tetrachloride at 1,600 or 3,200 mg/kg bw to four nursing rats resulted in hepatic damage in the neonates 24 or 48 hours later. A dose of 800 mg/kg bw to dams did not produce any hepatic damage to offspring.

Reproductive Effects

Testicular degeneration was observed in rats receiving carbon tetrachloride at 4,800 mg/kg bw intraperitoneally (frequently unstated) (Chatterjee, 1966). One group of six male rats received carbon tetrachloride as a 1:1 mixture in coconut oil; a control group received only an equal volume of coconut oil. On day 15 all animals were sacrificed. Body weights were similar for treated and control animals. However, the relative testes weight decreased from 15.5 (± 0.4) g/kg bw in controls to 9.8 (± 1.2) g/kg bw in exposed animals. Relative weight of seminal vesicles showed an even more pronounced decrease: 1.27 (± 0.171) g/kg bw in treated as compared to 3.10 (± 0.059) g/kg bw in control animals. Relative pituitary weight was also increased: 50 (± 1.4) mg/kg bw in treated as compared to 32.4 (± 0.9) mg/kg bw in control animals.

Histological examination of testes showed testicular atrophy and "some abnormality" in spermatogenesis in carbon tetrachloride-treated animals. The authors proposed a mechanism for carbon tetrachloride-induced testicular atrophy in which blockage of pituitary hormone release results in atrophy of Leydig cells within the seminal vesicles, followed by an abnormal spermatogenesis.

Intraperitoneal administration to male rats of carbon tetrachloride (4,800 mg/kg bw as a 1:1 mixture of coconut oil) for 10, 15, or 20 days (Group I, II, or III) led to impairments in spermatogenesis as indicated by histological examination (Kalla and Bansal, 1975). Controls were administered equal volumes of coconut oil. Weights of testes, seminal vesicles, epididymis and prostates were decreased in exposed animals, whereas the weight of adrenal glands increased (see Table V-6). The gonadosomatic index (GSI) (equal to body weight x testes weight/100) was also decreased in treated animals. A slight decrease in pituitary weight was observed following a 10-day treatment but not after either the 15- or 20-day treatment. As Table V-5 shows, the ratio of germinal to nongerminal area steadily decreased from Group I to Group III and was always higher in treated than control animals. Significant differences in total germinal area between treated and control animals, however, were observed only at 20 days. Histological examination did not reveal any abnormalities in testes from Group I. Clusters of mature sperm were present in the lumen. In Group II, slight testicular damage was observed; a decrease in spermatogenic cells and increased lumen size. In Group III, shrinkage of the tubules and increased area of the lumen were observed. Arrangement of the germ cells was disrupted; early gonadal cells were present in the lumen of many of the tubules. No spermatids were observed. Intersititial material was "damaged" and in many places the basement membrane was detached from the epithelium.

Thus, carbon tetrachloride, at a total dose of 48 g/kg bw over 10 days, had a distinct but minor effect on male rat reproductive physiology, whereas a total dose of 96 g/kg bw over 20 days resulted in severe disturbances of spermato genesis.

Table V-6 Weight Changes in Male Reproductive Organs After Carbon Tetrachloride Treatment

Treatment and Period	Body weight(g)		Testis (g/kg bw)	Seminal vesicles (g/kg bw)	Epididymis (g/kg/bw)	Prostate (g/kg/bw)	Adrenal (g/kg/bw)	GSI
	Before treatment	After treatment						
Group I 10 days								
Control	257±5[a]	269±4.5	12.36±0.94	4.25±0.55	4.45±0.45	2.1±0.7	0.18±.02	7.94±0.45
Treated	257±13.71	247±10.76	8.84±1.04	2.41±0.14	2.88±0.38	1.26±0.24	0.24±0.03	5.68±0.31
Group II 15 days								
Control	230±15.49	235±5.0	10.47±0.59	3.9±0.52	4.12±0.18	2.19±0.31	0.22±0.01	6.02±0.21
Treated	230±15.49	173±13.42	9.46±0.33	1.17±0.26	2.63±0.18	1.68±0.13	0.32+0.05	5.44±0.28
Group III 20 days								
Control	234±5.5	235±6.5	11.37±0.06	3.24±0.31	3.72±0.09	1.78±0.04	0.12±0.01	6.64±0.23
Treated	230±3.5	210±0.5	9.90±0.65	1.53±0.28	2.52±0.22	1.29±0.01	0.25±0.02	5.69±0.38

[a] Mean ± standard deviation.

Adapted from Kalla and Bansal (1975).

Mutagenicity

There have been no reports of mutagenic activity associated with carbon tetrachloride in any of the various Salmonella (Ames) assays. However, mutagenic activity associated with carbon tetrachloride has been reported in a eukaryotic test system using the yeast *Saccharomyces cerevisiae*. This recent report has not been confirmed and should not be accepted as conclusive evidence of carbon tetrachloride mutagenicity. Nevertheless, these eukaryotic test systems can be used to screen for mutagenic chromosomal effects that cannot be detected in prokaryotic systems. Callon *et al*. (1980) have suggested that the yeast system is more sensitive than the Salmonella assay system because active metabolites are produced in much closer priximity to the nucleus than they are with liver S-9 *in vitro* activation systems used in bacterial assays. Proximity to the nucleus would be advantageous if the active metabolites were particularly unstable or reactive, as is likely to be the case for those of halogenated hydrocarbons such as carbon tetrachloride.

Callon *et al*. (1980) reported that carbon tetrachloride, in addition to six other hydrocarbons, induced a mutagenic response in *S*. *cerevisiae* strain D7. Unlike the Salmonella system, in which promutagens must be treated with exogenous liver S-9 activation systems, the D7 strain of *S*. *cerevisiae* contains an endogenous cytochrome P-450 dependent mono-oxygenase activation systems. In addition, strain D7 can be used to detect both gene crossover and mitotic recombination. Three concentrations of carbon tetrachloride (21, 28, and 34 mM) were incubated at 37°C for either 1 or 4 hours in 3-ml aliquots of the yeast cell suspension (3×10^7 cells/ml). Treatment was terminated by the addition of 40 ml of ice-cold buffer followed by centrifugation to remove the cells from the media. The resuspended cells were then plated on appropriate media to allow for estimation of mutagenic activity. A spectral analysis of the cell suspension demonstrated that carbon tetrachloride was gradually altered during the incubation periods; the authors concluded that it was absorbed and metabolized by the cells.

The 1-hour treatment of cells with carbon tetrachloride resulted in significant increases in gene crossover and mitotic recombination. Results for the different loci are presented in Table V-7. When cells were treated with increasing carbon tetrachloride concentrations there was decreased cell survival and increased incidence of gene conversion and mitotic recombination. There was a nonlinear correlation between cell survival and the concentration of carbon tetrachloride in the incubation mixture. Only marginal mutagenic activity was noted when incubation was continued for 4 hours (data not reported by authors). This observation could have resulted from toxicity masking the genetic effects, the continued metabolism of active metabolites to inactive products, or the destruction of the metabolic system. Callon _et_ _al_. (1980) noted that carbon tetrachloride had been reported to be inactive in test systems which used an exogenous mammalian activation system and suggested that their yeast system was more sensitive than some of the other _in_ _vitro_ test systems.

TABLE V-7 Mutagenic Effects of Carbon Tetrachloride on Strain D7 of *Saccharomyces Cerevisiae*[a]

	Concentration (mg/liter)			
	0	3234	4312	5128
Survival				
Total colonies	1454	1252	1120	152
% of control	100	86	77	10
trp 5 locus (gene conversion)				
Total convertants	285	331	350	506
Convertants/10^5 survivors	2.0	2.6	3.1	61.7
ade 2 locus (mitotic recombination)				
Total twin spots	1	3	3	10
Mitotic recombinants/10^4 survivors	1.6	5.3	5.8	40.1
Total genetically altered colonies	11	19	16	65
Total genetically altered colonies/10^3 survivors	1.7	3.4	3.1	33.3
ilv 1 locus (gene reversion)				
Total revertants	38	41	57	11
Revertants/10^6 survivors	2.6	3.3	5.1	7.2

[a] The total number of colonies in the different classes represent total counts of colonies from five plates in the case of survival, conversion, and revertant-frequency estimations. Mitotic recombination was estimated from counts of colonies growing on a total of 30 plates, 20 plates containing medium of which all surviving cells grew and 10 plates containing medium on which only trp 5 convertants grew.

Adapted from Callen *et al*. (1980)

Studies to determine the mutagenic activity of carbon tetrachloride in the Salmonella typhimurium system have been uniformly negative (see reviews: McCann et al., 1975; Fishbein, 1976; and Rinkus and Legator, 1979). Carbon tetrachloride has also been void of mutagenic activity when tested in Escherichia coli (Uehleke et al., 1976).

Carbon tetrachloride did not induce chromosome damage (i.e., chromatid gaps, deletions, or exchanges) during an in vitro chromosomal assay using cultures rat-liver cells (Dean and Walker, 1979). Mirsalis and Butterworth (1980) found that treatment of adult male Fischer-344 rats (200-250 g) with carbon tetrachloride (10 or 100 mg/kg, po) produced no increase in unscheduled DNA synthesis in cultures of primary rat hepatocytes. According to the authors these results indicate that carbon tetrachloride does not act through a genotoxic mechanism and confirm the report of Craddock and Henderson (1978) that this chemical does not induce DNA repair in hepatocytes immediately following treatment.

Uehleke et al. (1977) studied the interaction of carbon tetrachloride in a liver microsome system used in conjunction with assays in S. typhimurium strains TA 1535 (test for base pair substitutions) and TA 1538 (tests for frame shift mutations). The authors incubated ^{14}C-labeled carbon tetrachloride (1 mM) with rabbit liver microsomes pretreated with phenobarbital (5 mg of protein/ml) and a NADPH regenerating system at 37°C for 60 minutes. A total of 10% of the radioactivity from carbon tetrachloride was irreversibly (covalently) bound to endoplasmic portein and more than 30% was bound to microsomal lipid. No mutagenic activity was observed in S. typhimurium strains TA 1535 or TA 1538 which were incubated with 8 mM carbon tetrachloride and microsomal suspensions. The authors concluded that a reactive species generated in the biological system may not distribute into the incubation medium and thus may be inaccessible to the test bacteria. They also speculated that the active products of carbon tetrachloride may have very short half-lives.

Carcinogenicity

The carcinogenic effects of carbon tetrachloride have been well documented (IARC, 1979). IARC has judged the evidence from animal studies demonstrating that carbon tetrachloride induced hepatic neoplasms as conclusive for experimental animal carcinogenesis (IARC, 1979).

In a NCI bioassay program, carbon tetrachloride was used as a positive control in the bioassays of chloroform and 1,1,1-trichloroethane (NCI 1976, 1977). The positive control groups described in both bioassays were of the same strain and source as the treated animals and were housed identically. Groups of 50 Osborne-Mendel rats of each sex were administered carbon tetrachloride in corn oil by gavage five times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg bw for males, 80 and 160 mg/kg bw for females. This treatment resulted in toxicity: at 110 weeks at the highest dose, only 7 of 50 males and 14 of 50 females survived as compared to 26 of 100 males and 51 of 100 females for controls. The incidence of hepatocellular carcinomas was increased in animals exposed to carbon tetrachloride as compared to controls (see Table V-8). Absolute incidence of hepatic neoplasms was low. The apparent decrease in the incidence of hepatocellular carcinomas in female rats at the high dose was attributed to increased lethality, i.e., females died before tumors could be expressed. The incidence of other neoplasms in these rats was acknowledged but not quantified.

Table V-8. Incidence of Liver Tumors in Carbon Tetrachloride-Treated Rats and Colony Controls

Animal group		Hepatocellular carcinoma	Neoplastic nodule
Males	Controls	1/99	0/99
	Low dose	2/50	2/50
	High dose	2/50	1/50
Females	Controls	0/98	2/98
	Low dose	4/49	2/49
	High dose	1/49	3/49

Source: NCI (1976)

Groups of 50 B6C3F1 mice of each sex were administered carbon tetrachloride in corn oil by gavage five times weekly for 78 weeks at two doses, 1,250 and 2,500 mg/kg bw, for both males and females. High exposure-related lethality occurred in all groups. At 78 weeks at the high dose, only 2 of 50 males and 4 of 50 females survived as compared to 53 of 77 V-36 males and 71 of 80 females for controls. At 91-92 weeks at the high dose, none of 50 males and 1 of 50 females survived as compared to 38 of 77 male and 65 of 80 female controls. An exposure-related increase in the incidence of hepatocellular carcinomas was observed (see Table V-9). The average latency for appearance of liver tumors was also significantly decreased in carbon tetrachloride exposed animals. In high-dose males, the latency was 26 weeks, as compared to 48 weeks in low-dose males and 72 weeks in control males. In high-dose females, the latency was 19 weeks, as compared to 16 weeks in low-dose and 90 weeks in control female mice.

TABLE V-9 Comparison of Hepatocellular Carcinoma Incidence in Carbon Tetrachloride-Treated Mice Vehicle-Treated Controls

Animal group		Hepatocellular carcinoma
Males	Controls	5/77
	Low dose	49/49
	High dose	47/48
Females	Controls	1/80
	Low dose	40/40
	High dose	43/45

Source: NCI (1976)

The comparative carcinogenicity of carbon tetrachloride has been studied in five rat species: Japanese, Osborne-Medel, Wistar, Black, and Sprague-Dawley (Reuber and Glover 1970). Groups of 12-17 male rats of each strain were given twice weekly subcutaneous injections of carbon tetrachloride (2,080 mg/kg bw as a 50% solution in corn oil). Treated animals were sacrificed when moribund; controls for each strain were sacrificed at the time as the last experimental animal. Incidence of hepatic lesions is given in Table V-10. The data indicate that: (i) sensitivity to carbon tetrachloride-induced neoplasms varies widely among strains; and (ii) the trends in incidence of neoplasms and cirrhosis run exactly opposite. Varying amounts of toxicity occurred. all experimental animals of the Black rat strain were dead at 18 weeks, and those of Sprague-Dawley Strain at 16 weeks; the failure to find carcinomas in those strains may have been caused in part by an insufficient latency time. In all three other strains, significant toxicity (i.e., lethality) occurred. Toxicity decreased in the same order as carcinogenicity increased. Thus, it appears that there is no causal connection between the degree of toxicity and carcinogenicity.

Other neoplasms were also observed, all in the Osborne-Mendel and Japanese strains. (It is unclear from the text whether these were in experimental or control animals.) Heman-giomas of the spleen were seen in three rats: two Japanese and one Osborne-Mendel. Six carcinomas of the thyroid gland were observed: Three in Japanese and three in Osborne-Mendel rats. Multicystic kidneys were observed in two Osborne-Mendel and three Japanese rats. One rat of the Japanese strain had a subcutaneous leiomyosarcoma.

Relative organ weights were decreased for testes and increased for liver, spleen, and kidneys of all experimental animals as compared to strain controls. The extent of atrophy of the testes, prostate, and seminal vesicles was correlated with the degree of cirrhosis.

The carcinogenicity of carbon tetrachloride in hamsters has also been described (Della Porta et al., 1961). Groups of 10 Syrian golden hamsters of each sex were administered weekly by gavage 20 mg of carbon tetrachloride (5% solution in corn

TABLE V-10. Evidence of the Most Advanced Lesions In Rats Administered Carbon Tetrachloride

	Japanese	Osborne-Mendel	Wistar	Black	Sprague-Dawley
No hyperplasia	0/15	0/13	0/12	4/17	8/16
Hyperplasia	0/15	1/13	1/12	6/17	6/16
Hyperplastic nodule	3/15	4/13	7/12	7/17	2/16
Small carcinoma	4/15	4/13	3/12	0/17	0/16
Large carcinoma	8/15	4/13	1/12	0/17	0/16
Total carcinoma	12/15	8/13	4/12	0/17	0/16
No cirrhosis	0/15	0/13	0/12	0/17	0/16
Mild cirrhosis	9/15	2/13	0/12	0/17	0/16
Moderate cirrhosis	5/15	7/13	6/12	4/17	0/16
Severe cirrhosis	1/15	4/13	6/12	13/17	16/16

Adapted from Reuber and Glover (1970)

oil) for 7 weeks, followed by 10 mg for 23 weeks (equivalent to 200 and 100 mg/kg bw). Survivors were sacrificed at 55 weeks. Postnecrotic cirrhosis was observed in all animals that died in week 41. Postnecrotic cirrhosis was described by the authors to involve "regenerative hyperplastic nodules." In current experiments, these probably would have been described as neoplastic nodules in view of the following micropathological findings: nodules had obliteration of normal lobular architecture and were surrounded by fibrous tissues; cells were irregular in shape and size; nuclei and cytoplasm stained abnormally with uneven distribution of glycogen. Each of the other 10 animals had one or more hepatic carcinomas. A total of 22 neo-plasms was observed: 12 in five females and 10 in five males. Some were sizeable, measuring 5-30 mm.

Thus, Syrian golden hamsters appear sensitive to the carcinogenic effects of carbon tetrachloride. Although the number of animals in this study was small, the authors considered the results to be significant because the reported control rate of hepatic tumors in hamsters were 0/254.

Carbon tetrachloride was also reported to be carcinogenic in C3H mice (Andervont, 1958). Groups of 30-77 female or male C3H mice were administered by gavage 6.46 mg of carbon tetrachloride once weekly for 2 weeks, followed by administration of 9.6 mg once weekly for 17 weeks (equivalent of 213 and 320 mg/kg bw). Pathogen-free or normal C3H mice were used. For no difference in the incidence of hepatomas was observed between pathogen-free and normal mice: 79% as compared to 49% in controls. The average number of hepatomas per animal was 1.8 in treated animals and 1.3 in controls. In females, a difference between the incidence and hepatomas in pathogen-free and normal rats was observed: 46% and 29%, respectively as compared to 3% in controls. The average number of hepatomas per mouse was 1.5, 1.2, and 1.0, respectively, indicating that both the incidence as well as the average number of tumors per animal increased in the order: controls <treated normal mice <treated pathogen-free mice.

In another study, liver necrosis and hepatomas were noted after NCI strain A mice were treated with carbon tetrachloride for 4 months (Eschenbrenner and Miller, 1946). Groups of five male or female mice were administered carbon tetrachloride by gavage at 0, 1,200, 2,400, 4,800, or 9,600 mg/kg bw for either 120 doses in 119 days (120/119) or 30 doses in 116 days (30/116) and sacrificed 30 days after cessation of administration. The incidence of hepatomas and necrotic lesions is noted in Table V-11. At the three highest doses on the 120/119 dose schedule, a 100% incidence of hepatomas was observed. At the highest dose, the mice had numerous hepatomas of up to 1 cm in diameter. At the two intermediate doses the neoplasms were less frequent and smaller in size. No hepatomas were observed in the 1,200 mg/kg group. The incidence of hepatomas was decreased in the animals on the 30/116 schedule as compared to V-42 those on the 120/119 schedule. At the 1,200 mg/kg dose, however, very small hepatomas were detected by microscopic examination in two males. The authors concluded that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with carbon tetrachloride.

A third study also reported the induction of hepatomas in mice by exposure to carbon tetrachloride (Edwards, 1941). Of 143 female C3H mice administered 64 mg of carbon tetrachloride two or three times weekly for 36-55 doses, 126 or 88.1% developed hepatomas. In similar experiments, a 100% incidence of hepatomas was observed in 54 male and female strain A mice having received 23-58 doses. The first hepatoma was observed following 23 doses.

Summary

Carbon tetrachloride-induced hepatic effects have been reported after both acute and chronic exposure. The degrees of toxicity and of hepatic damage have appeared to be dose-related, as measured by liver enzyme activity in at least four species: mouse, rat, guinea pig, and dog. In parallel acute studies in mice and dogs in which dose-response relationships for minimal toxic effects were developed, mice have appeared more sensitive to the toxic effects of carbon tetrachloride than have dogs. Liver damage from acute exposure to carbon tetrachloride has been reported to be reversible. At 36 hours after acute exposure to various doses of carbon tetrachloride, histopathological examination has shown no abnormalities in liver specimens. In chronic exposure, liver damage has been reported to

Table V-11 Susceptibility of Strain A Mice to Liver Necrosis and the Incidence of Hepatomas 30 Days After 120 or 30 Doses of Carbon Tetrachloride[a]

		Carbon tetrachloride dose[b]														
		9600 mg/kg			4800 mg/kg			2400 mg/kg			1200 mg/kg			0 (olive oil)		
Sex	Dose Conditions (doses/days)	Probable initial liver necrosis	Observed final liver necrosis	Hepatoma incidence	Probable initial liver necrosis	Observed final liver necrosis	Hepatoma incidence	Probable initial liver necrosis		Observed final liver necrosis	Hepatoma incidence	Probable initial liver necrosis	Observed final liver necrosis		Hepatoma incidence	Probable initial liver necrosis
F	120/119	+	0/5	5/5	+	0/5	5/5	±	0/5	5/5	–	0/5	0/5	–	0/5	0/5
M	120/119	+	0/4	4/4	+	0/5	5/5	±	0/5	5/5	–	0/5	0/5	–	0/5	0/5
F	30/116				+	2/4	2/4	+	2/5	3/5	+	0/5	0/5	–	0/5	0/5
M	30/116				+	4/4	3/4	+	3/5	4/5	+	0/5	$0/5^c$	–	0/5	0/5

[a] Based on gross and microscopic examination; [b] Cumulative dose

[c] Two animals showing no tumor on gross examination were found each to have a very small hepatoma on microscopic examination of a random section of the livers.

lapted from Eschenbrenner and Miller (1946).

be reversible if the damage did not advance to the necrosis stage.

The degenerative liver changes observed in both acute and chronic exposure have been increased serum/plasma levels of hepatic enzymes, progressing through more pronounced cellular degenerative changes such as fatty liver and proliferation of rough endoplasmic reticulum, to fatty liver degeneration and necrosis. Lung damage has included similar cellular effects, with the majority of changes occurring within the Clara cells. Degenerative kidney effects have also been observed, but appeared significant only after high doses of carbon tetrachloride.

Carbon tetrachloride has produced prenatal toxic effects, which could not be well correlated with extent of maternal exposure. Rats exposed to carbon tetrachloride in utero have shown hepatic abnormalities at birth, but the lifetime effects of these changes were not reported.

Carbon tetrachloride has produced distinct degenerative changes in testicular histology, eventually resulting in aspermatogenesis and functional male infertility. These effects occurred at medium to high doses.

Carbon tetrachloride has elicited a mutagenic response in a Saccharomyces cervisiae test system, but has consistently tested nagative in the Salmonella (Ames) assay. Investigators have attributed the failure of carbon tetrachloride to induce a mutagenic response in Salmonella to the absence of an in vivo activating system in the prokaryote. No other mutagenic responses to carbon tetrachloride have been reported in the literature.

Carbon tetrachloride has been reported to be carcinogenic in numerous animal studies. Hepatocellular carcinomas have been the neoplasm induced in all species. Hamsters have been the most sensitive species studied, followed by mice and then rats. A significant strain difference has been observed in rats. Females have appeared less sensitive to the chronic toxic effects and more sensitive to the carcinogenic effects of carbon tetrachloride in both rats and mice.

VI. HEALTH EFFECTS IN HUMANS

Considerable human exposure to carbon tetrachloride through inhalation has come through its use as an industrial solvent and dry cleaning fluid. Ingestion of carbon tetrachloride or mixtures containing carbon tetrachloride has also been documented in various case reports. Ingestion has occurred under different circumstances (e.g., suicide attempts, medical use) by persons of diverse occupations and ages. These acute exposures have been followed by hepatoxic effects accompanied by acute nephrosis.

In the following section the effects of carbon tetrachloride exposure are presented as reported in case studies and in controlled studies for humans. The case studies are divided into reports of acute and long-term effects. The human case studies are often anecdotal, with missing or imcomplete medical descriptions of clinical signs of poisonings. In the controlled studies using human volunteers, changes in serum and urine chemistry were measured after exposure to carbon tetrachloride, but no histologic specimens were taken. Because of the limitations in these studies of humans, they are presented as supporting evidence for the harmful effects of carbon tetrachloride in humans.

Case Studies--Acute Effects

Ingestion. Lamson et al. (1928) studied the lethal effects of carbon tetrachloride in patients receiving carbon tetrachloride and magnesium sulfate orally as a treatment for hookworms. The authors reported the treatment of thousands of patients with a single dose of 2.5-15 ml of carbon tetrachloride without ill effects. One man was reported to have safely ingested 40 ml of carbon tetrachloride. However, an "extremely small" population of adults died after receiving 1.5 ml of carbon tetrachloride; doses of 0.18-0.92 ml were reported to be fatal to children. Susceptibility in adults was correlated with alcoholic intake (chronic alcoholism or exposure to alcohol shortly after treatment), the presence of ascarid worms, and the intake of foods, particularly of high fatty content.

A fatality attributed to ingestion of carbon tetrachloride was reported by Smetana (1939). The victim, a photographer described as having "a history of chronic alcoholism," died 10 days after consuming an unknown amount of "some fluid containing carbon tetrachloride." He presented symptoms including nausea, vomiting, jaundice, anuria, and semistupor. In the final clinical diagnosis, death was attributed to carbon tetrachloride poisoning.

A case of attempted suicide by ingestion of carbon tetrachloride was reported by Stewart et al. (1963). The victim, a 29-year-old female who ingested 1 pint of a carbon tetrachloride: methanol solution (2:1), experienced ringing in the ears immediately after ingestion and lost consciousness. She was hospitalized for 3 weeks. Three hours after ingestion, carbon tetrachloride in the exhaled breath and blood was confirmed by infrared analysis. The exhaled breath was then monitored throughout the hospitalization, and was reported to decrease exponentially. Because of the toxicity of the methanol and the possibility of synergistic reactions with the carbon tetrachloride, hemodialysis was performed soon after admission. Mannitol solution was given by continuous intravenous infusion. Clinical laboratory analyses during hospitalization showed some elevation of SGOT, which reached a maximum of 75 units at day 6, and an elevation of urinary urobilinogen to a maximum of 7.8 Ehrlich units at day 10. Other laboratory findings included elevation of serum iron and depression of serum protein concentration and albumin fractions. The retention time of bromosulfophthalein was increased. These finding were interpreted as evidence of minimal hepatocellular injury. Acute renal dysfunction was not observed; the authors credited the mannitol treatment with preventing renal damage.

Acute Effects

Inhalation. Bilateral peripheral constriction of the ocular color fields, resulting in symptoms of toxic amblyopia in three males, was attributed to the inhalation of carbon tetrachloride vapors (Wirtschafter, 1933). Five male employees of dry cleaning establishments who had been exposed to carbon tetrachloride (of unknown concentration) from 8-10 hours daily for 1-6 months were examined. Two men also had signs of conjunctivitis. Three of the men complained of visual disturbances characterized by blurred vision or spots before the eyes. The author concluded that toxic amblyopia may result from exposure to carbon tetrachloride vapor.

One fatality occurred in two cases of carbon tetrachloride poisoning reported by Smetana (1939). In the fatality, a dry cleaner and interior decorator described as being "a steady and heavy drinker" was exposed for several hours to carbon tetrachloride vapors during work. Upon returning from work, he noted dyspnea. Several hours after the exposure, headache, dizziness, and malaise developed, accompanied by nausea and repeated vomiting that persisted for several days. The patient also suffered labored breathing and cough with bloody sputum before he died 9 9 days following exposure.

The second inhaltion case reported by Smetana was a housemaid also described as having a history of chronic alcoholism. Three days before hospitalization, the patient cleaned dresses with carbon tetrachloride for 3 hours in a poorly ventilated room. Soon after exposure, she began to vomit. She suffered symptoms similar to those described for the other case. After approximately 1-1/2 months of hospitalization, this patient was released from the hospital her condition several weeks later was described as "much improved."

Seven cases of carbon tetrachloride poisoning reported by Norwood et al. (1950) resulted from both occupational and non-occupational inhaltion exposures. In the three cases described as "severe" poisonings, there was a history of chronic alcoholism; two fatalities occurred in this group. In one case, the victim had been exposed for about 15 minutes to an atmosphere containing carbon tetrachloride at an estimated 1,575 mg/m^3 (this estimate was made by duplicating the conditions). Histopathologic examination of liver and kidney tissue from the fatalities revealed liver necrosis and degenaration of the renal tubules. The four remaining cases were characterized as "mild industrial" exposures. After exposure to carbon tetrachloride, all subjects suffered varied symptoms including nauseas, vomiting, diarrhea, headache, muscular ache, pain, or numbness, labored breathing, and dizziness.

In another case, a 31-year-old janitor suffered malaise, back and lower abdominal pain, nausea, and vomiting the morning after working for 5 hours in a closed room with carbon tetrachloride (Kittleson and Borden, 1956). He reportedly consumed two bottles of beer during the exposure period. The patient required 2 months of hospitalization for treatment of acute renal insufficiency as a result of carbon tetrachloride intoxication.

Elevated serum glutamic oxaloacetic transaminase (SGOT) activities with concomitant liver changes were reported in two men occupationally exposed to unreported concentrations of carbon tetrachloride (Lachnit and Pietschmann, 1960). One became ill after exposure to carbon tetrachloride for 3 hours in a relatively well-ventilated room. He was hospitalized 3 days after exposure. His liver was slightly enlarged, with the (SGOT) value elevated by 6,000 units. This value rapidly decreased and returned to normal by the 10th day. A biopsy of the liver taken on the 8th day showed necrosis in the centers of the lobuli, but the surrounding tissue was undamaged. An additional needle biopsy of the liver taken at the 28th day showed that the cells had almost returned to normal. In the second case a male similarly exposed to carbon tetrachloride entered the hospital 12 days after exposure. The SGOT had increased to 80 units. A liver needle biopsy on the 22nd day showed only moderate changes, some of a degenerative nature.

In a chemical packing plant, use of carbon tetrachloride by two workers for equipment cleaning, as a substitue for the customarily used acetone, resulted in the hospitalization of 4 of 43 workers at the plant (Folland et al., 1976). Ten additional workers also became ill. Eight of the 43 workers fell ill within 12 hours following the start of the 2-hour exposure; six others followed within the next 36 hours. The four hospitalized workers showed evidence of severe disruption of liver function: one case had an SCOT level of 13,390 units. All patients recovered within 90 days. All hospitalized workers as well as most of the others taken ill had worked near a bottle-filling operation for isopropyl alcohol at the northern end of the plant, adjacent to the carbodn tetrachloride cleaning area.

Carbon tetrachloride concentrations at the time of exposure were not ascertained; acetone was normally used for cleaning. Isopropyl alcohol concentrations at the northern end of the plant average 410 ppm. Acetone in alveolar air samples of workers in the northern area averaged 19 ppm. The authors described the toxic episode to carbon tetachloride toxicity potentiated by isopropyl alcohol. Because carbon tetrchloride concentrations were unknown and isopropyl alcohol (and possibly other chemicals) were present, the health effects reported in this study cannot be attributed to carbon tetrachloride exposure alone.

Case Studies Long-Term Effects

Straus (1954) suggested a possible causal relationship between carbon tetrachloride exposure and aplastic anemia. Three males had been exposed to carbon tetrachloride at unknown concentrations for 2 months to 3 years. Autopsy findings included hypoplasia of the bone marrow. However, a causal relationship between carbon tetrachloride and aplastic anemia suspected by the author in these cases is not supported adequately. One of the men had also been exposed to kerosene for 3 years. Another was an auto mechanic who worked in a garage. The occupation of the third was not specified although his exposure to carbon tetrachloride was occupationally related. Thus the effects of other chemicals cannot be discounted. The autopsy findings of two of the patients included no liver or kidney damage of the type that would be expected in carbon tetrachloride poisoning. In one case the liver was reported to have toxic hepatitis which was considered to be the result of carbon tetrachloride poisoning. The information reported in these case studies tends not to substantiate the author's suggestion that the patients' illnesses may have been caused by carbon tetrachloride.

Carcinogenicity The possibility of carcinogenic effects of carbon tetrachloride in humans has been raised in a number of case reports. These reports do not establish a causal link between carbon tetrachloride exposure and the incidence of neoplasms (heptomas). Thus, the suggestion that carbon tetrachloride is carcinogenic in humans remains purely speculative.

A 59-year-old man with a history of moderate alcohol consumption returned from a cocktail party and noticed the vapor of carbon tetrachloride used to clean a rug in his apartment earlier that evening. Five days later he developed nausea, vomiting, and diarrhea and within 10 days of exposure he developed jaundice (Tracey and Sherlock, 1968). The patient recovered following a long and complex hospitalization and was discharged after 9 weeks. Four years after hospitalization for jaundice, he was found to have a smooth, enlarged, nontender liver. He denied alcohol consumption within the intervening period. Three years after this checkup, the patient was readmitted with a history of nausea, vomiting, and diarrhea. A liver biopsy was diagnosed as hepatocellular carcinoma. No treatment was administered until readmission 5 months later when he received X-ray radiation dose of 3000 R. He died 2 weeks after discharge. Postmortem examination revealed the liver to be extensively involved with the tumor. Little normal liver tissue remained.

The connection between carbon tetrachloride exposure, potentiated by prior alcohol use, and the induction of jaundice appears well established. In contrast, as the authors state, no causal relationship between the carbon tetrachloride exposure and hepatocellular carcinoma can be drawn from this report. Aside from the acute exposure to carbon tetrachloride 7 years before diagnosis of cancer, the patient's possible additional exposure to this and other toxic chemicals was not reported. No medical history was given for the 3 years before the final diagnosis.

A study of residents of an area surrounding a solvent recovery plant in rural Maryland found a great increase in the incidence of lymphatic cancer (Capurro, 1979). The mortality experience of residents of a I.5 km^2 area around the plant--which emitted at least 31 chemicals (identified by gas chromatography), including carbon tetrachloride--was followed from October 1968 to October 1974. Six deaths, one due to cancer, were expected over this period. Fourteen deaths were observed, including seven from cancer. Four of seven malignancies were lymphomas, more than 60 times the expected incidence. These deaths were not attributed to any particular chemical.

Other case reports of human neoplasms developing after exposure to carbon tetrachloride have appeared. In one case a woman developed modular cirrhosis of the liver followed by cancer of the liver after exposure to carbon tetrachloride, and died 3 years after the first exposure (Johnstone, 1948). However; she had suffered from periodic jaundice for 5 years prior to exposure to carbon tetrachloride. In a second case, a fireman developed cirrhosis and an "epithelioma" of the liver 4 years after acute carbon tetrachloride intoxication (Simler et al., 1964). In none of the cases could a causal link between carbon tetrachloride exposure and development of neoplasms be established.

Because of concomitant exposure to other chemicals this study does establish a causative association between carbon tetrachloride exposure and increased mortality.

Epidemiology. A retrospective study of laundry and dry cleaning workers was conducted by Blair et al. (1979) to determine if occupational exposure to carbon tetrachloride, trichloroethylene, tetrachloroethylene, and petroleum solvents resulted in increased morbidity or mortality. Data for cases were obtained from union records benefits lists. Sex, race, age at death, and and underlying and contributing cause of death were abstracted from death certificates. The age, race, sex, and cause distribution for all deaths in the United States from 1957-1970 served as the control standard. Causes of death were analyzed using the proportionate mortality method. The results of analysis demonstrated an excess of lung and cervical cancer, and slight excesses of leukemia and liver cancer.

Because of the multiple historical exposures experienced by this population, it is difficult to establish a causal association between specific substances and increased mortality trends. This study is presented as evidence of a possible link between carbon tetrachloride exposure and increased mortality rather than as a study in which quantitative extrapolations of the chemical's effects on human health are possible.

A cross-sectional epidemiologic study (Sonich et al., unpublished) examined health effects of CCl_4 ingestion in humans. Seventy tons of carbon tetrachloride were spilled in the Kanawha and Ohio River in 1977. Measurements of raw water revealed maximum concentration of 0.340 mg/l. Twenty-one cities situated along the river were involved in the study. These cities represent areas that obtained their drinking water directly from the river and/or area that obtained their drinking water from sources not influenced by the quality of the river water. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to carbon tetrachloride were estimated for each city along the river. The results of routine tests measuring serum chemistries reflecting liver and kidney function along with basic epidemiologic information were abstracted from approximated 6,000 medical records. The results obtained for creatinine show a positive and statistically significant ($p<.05$) relationship between the carbon tetrachloride exposure and the frequencly of elevated levels of serum creatinine in exposed patients. No similar results were found for the other parameters analyzed.

Controlled Studies

Inhalation. Human volunteers were exposed to known concentrations of carbon tetrachloride vapor in an effort to correlate physiological and/or biochemical changes to the magnitude of exposure (Stewart et al., 1961). Eight healthy male volunteers were exposed to carbon tetrachloride vapors in a series of three separate experiments performed 1 month apart. Prior to exposure, data on blood pressure, SGOT, and urinary urobilinogen were obtained for each subject. Samples of pre-exposure exhaled breath, urine, and blood showed no detectable carbon tetrachloride. The volunteers were seated in a closed room (11 x 12 x 7.5 feet) where 99% pure carbon tetrachloride was poured into a dish and covered with a towel. An exhaust system grill and door were closed during the experiment but an air supply grill was left open. A fan circulated air across the dish. Carbon tetrachloride ambient concentrations were monitored with a Davis halide meter and an infrared spectrameter. The carbon tetrachloride concentration ranges and exposure times are given below in Table VI-1.

TABLE VI-1 Exposure Times and Concentrations of Carbon Tetrachloride Vapor in a Study by Stewart et al. (1961)

Experiment	Average concentration, time-weighted (mg/m^3)	Concentration range	Exposure (minutes)
1	309	192-548	70
2	69	63-88	180
3	63	57-88	180

Carbon tetrachloride was detected in exhaled breath in all three experiments. Graphs showed an exponential decrease in concentration of carbon tetrachloride versus time. The exact values were not given.

The serum iron showed an initial decrease in three of six subjects at the 309 mg/m^3 exposure level but had returned to normal in two of these subjects 68 hours after exposure. The remaining subject showed a 31% depression in serum iron at 68 hours, but the value was within the normal range. Serum iron was not analyzed in the other two experiments. Of the six subjects exposed to carbon tetrachloride at 309 mg/m^3, the serum transminase level was slightly elevated in some and depressed in others, but remained within the normal range. Carbon tetrachloride was not detected in the blood or urine at any exposure time or dose, but the analytical technique used was not a sensitive one. The authors concluded that no ill effects were observed from exposure to carbon tetrachloride at 63 mg/m^3 for 180 minutes, although the small changes in serum iron at the 309 mg/m^3 dose might have been an indication of liver insult.

Absorption through Skin. The absorption of carbon tetrachloride through human skin was measured by immersion of the thumbs of three male and female volunteers in a sample of this compound for 30 minutes (Stewart and Dodd, 1964). The carbon tetrachloride was analyzed by infrared spectroscopy, and no impurities were detected. Sequential sensations of burning and cooling were experienced by all volunteers during the immersion.

Burning ceased about 10 minutes after removal from the solvent. The thumbs of all volunteers appeared scaly and red, a condition that improved within several hours after exposure. Carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes of immersion of their thumbs. The concentration in the expired breath rose continuously to a maximum of 4.0 mg/m^3 10 to 30 minutes after the exposure period ended, and then decreased exponentially. The mean concentration of carbon tetrachloride was 2.0 mg/m^3, 2 hours after the end of exposure; at 5 hours after exposure, the alveolar air concentration was still greater than 0.6 mg/m^3. The authors concluded that carbon tetrachloride could be absorbed through the skin in toxic quantities.

Reproductive Effects. No teratogenic effects in humans caused by carbon tetrachloride exposure have been reported. However, human fetuses in one study appeared to have selectively accumulated carbon tetrachloride from the mother's circulation (Dowty *et al.*, 1976). Maternal blood samples were taken from 11 women either before or directly after (vaginal) delivery. (Prior exposure of the women to toxic chemicals was not reported.) Paired cord blood samples were obtained immediately after delivery. All volatiles were analyzed by gas chromatography and mass spectrometry. Carbon tetrachloride, benzene, and chloroform were present in higher concentratation in cord blood as compared to maternal blood.

Summary

Hepatic necrosis and renal pathology appear to be characteristic effects of acute human exposure to carbon tetrachloride. If exposure is terminated, the liver shows regeneration in most cases. In cases of acute renal dysfunction, kidney function returns to normal after exposure to carbon tetrachloride is terminated and medical treatment is given.

The possibility of an association between carbon tetrachloride exposure and cancer or aplastic anemia is not substantiated in epidemiological and case studies. These studies are limited in number, and all suffer from the confounding factor of exposure to multiple chemicals.

VII. MECHANISMS OF TOXICITY

The toxicity of carbon tetrachloride to an organism depends upon the ability of the organism to metabolize the compound. Thus, unmetabolized carbon terachloride does not appear to be significantly toxic (Rechnagel and Glende, 1973). In mammals, carbon tetrachloride is thought to be metabolized in the endoplasmic reticulum of the liver by the mixed-function oxidase system of enzymes. The reaction sequence proposed in the literature for carbon tetrachloride metabolism was outlined in Section III, Pharmacokinetics. Two free radicals have been postulated as metabolic intermediates: the trichloromethyl radical and the chlorine radical. The toxicity of carbon tetrachloride has been attributed to subsequent reactions of the trichloromethyl radical. These reactions include formation of carbonyl chloride (phogene), dimerizatiion to hexachloroethane, free radical binding protein, and lipid peroxidation. In this section each of these proposed pathways will be presented in conjuction with the toxic effects attributed to it.

Formation of Cabonyl Chloride (phosgene)

From the results of an *in vitro* study of carbon tetrachloride metabolism, Shah *et al*. (1979) postulated the formation of carbonyl chloride from the trichloromethyl radical. The authors incubated L-cysteine and [^{14}C] carbon tetrachloride with rat liver homogenate and looked for the formation of 2-oxothiozolidine-4-carboxylic acid. This compound is formed from the reaction of L-cysteine and carbonyl chloride. Analysis of the metabolic products by mass spectroscopy showed a fragmentation pattern consistent with 2-oxothiozolidine-4-carboxylic acid. The authors inferred from these analytical data that carbonyl chloride was formed as a metabolic product of carbon tetrachloride. Although carbonyl chloride (phosgene) is not reported to be a carcinogen, the authors pointed out that the compound is highly toxic and that the reactive chlorines could react with macromolecules in ways similar to alkylating agents.

Dimerization to Hexachloroethane

Hexachloroethane has been identified as a metabolite of carbon tetrachloride by Fowler (1969). The formation of this compound is believed to take place by the dimerization of the trichloromethyl radical. Although hexachloroethane is a hepatotoxin, its toxicity is less than that seen in carbon tetrahloride poisoning. Therefore, other mechanisms probably account for the severity of the toxicity associated with carbon tetrachloride.

Free Radical Binding to Proteins

Free radical binding to proteins had been postulated as one cause of toxicity associated with carbon tetrachloride (Rechnagle and Glended, 1973). The binding was reported to involve reactions with cellular proteins, particularly those with sulfhydryl groups. Experimental results have not confirmed this theory of carbon tetrachloride toxicity, nor is it supported by the pathological changes seen in the liver resulting from carbon tetrachloride exposure. In one study, [^{14}C] carbon tetrachloride has been observed to bind irreversibly to rabbit microsomal proteins at a rate of approximately 20 mole per mg of protein per hour (Uehleke and Werner, 1975). Binding of carbon tetrachloride (or its metabolites) to hepatic macromolecules was enhanced in the absence of oxygen, consistent with the proposal that the trichloromethyl radical is the reactive metabolite.

Although it has been shown that ^{14}C from carbon tetrachloride binds to proteins, the question of carbon tetrachloride binding to polynucleotides is still open. The question is important because of its implications for the mechanism of carbon tetrachloride carcinogenicity and mutagenicity. Rocchi _et al_. (1973) examined the possible binding of carbon tetrachloride with nucleic acids in Wistar rats and Swiss mice _in vivo_, and with DNA and polynucleotides _in vitro_. ^{14}C-labelled carbon tetrachloride (367 umol/kg) binds _in vivo_ to DNA of mouse liver and to ribosomal RNA of rat liver if the animals have been pretreated with 3-methylchloanthrene (MCA). The pretreatment with MCA increases the amount of the binding due to a higher activity of the microsomal system activating carbon tetrachloride. _In vitro_ carbon tetrachloride (0.218 umol) is activated by microsomes and pH 5 enzymes of MBA-treated animals to a metabolite which can react with DNA and polynucleotides. No binding of carbon tetrachloride metabolites to hepatic DNA from control mice or rats was detected. On the other hand, Uehleke and Werner (1975) incubated [^{14}C] carbon tetrachloride with either isolated liver microsomes (rat or mouse, species not identified) or with soluble RNA, they observed no [^{14}C] binding to ribosomal RNA or exogenous RNA. Experimental details were not presented.

Diaz Gomez and Castro (1980) reported that ^{14}C from carbon tetrachloride irreversibly binds *in vivo* to hepatic nuclear DNA from strain A/J mice and Sprague-Dawley rats (Table VII-1). Also binding of ^{14}C from carbon tetrachloride to DNA was observed *in vitro* in incubation mixtures containing microsomes and a NADPH generating system as well as in tissue slices (Table VII-2). Liver nuclear proteins (Table VII-3) and lipids (Table VII-4) irreversibly bind carbon tetrachloride metabolites. The authors concluded that: (a) the differences between the results of Rocchi et al. (1973) (pretreatment with MCA required) and theirs are possibly related to the use of different strains of mice (Swiss vs A/J) and rats (Wistar vs Sprague-Dawley); (b) the interaction of carbon tetrachloride metabolites with DNA and nuclear proteins could be relevant to carbon tetrachloride induced liver tumors and hepatoxic effects; and (c) the epigenetic mechanisms for chemical induction of cancer, not involving carbon tetrachloride-DNA interactions as shown in their study, could also be relevant.

Lipid Peroxidation

A number of the hepatic effects resulting from carbon tetrachloride exposure, including the fatty liver syndrome, are believed to arise as a result of lipid peroxidation (Rechnagel and Glende, 1973). The mechanism proposed for the peroxidation

TABLE VII-1 Covalent Binding of ^{14}C from $^{14}CCl_4$ to Rat and Mouse Liver DNA *In Vivo*[a]

Species	pmol/mg ± SD	^{14}C from $^{14}CCl_4$ in DNA[b] mol nucleotide/mol of CCl_4 metabolite (x 10^6)
A/J mice	0.72 ± 0.05	4.54
Sprague-Dawley	0.52 ± 0.05	6.25

[a] $^{14}CCl_4$(27 mCi/mmol) was administered ip as an olive oil solution (25 uCi/ml) at a dose of 10 ml of solution/kg bw. Animals were sacrificed 6 hr after $^{14}CCl_4$ and DNA was isolated and counted. For calculations it was considered that 1 mg of DNA contained 3,237 umol of nucleotides.

[b] Results are the mean of 3 samples. Each sample was a pool of 10 livers in the case of mice and one liver for rats. Values for rats were significantly lower than those of mice ($p<.05$).

Adapted from Diaz Gomez and Castro (1980)

TABLE VIII-2 Covalent Binding of ^{14}C from $^{14}CCl_4$ to DNA *In Vitro*

Experimental	^{14}C From $^{14}CCl_4$[b] DNA pmol/mg ± SD	 DNA mol nucleotide/mol of CCl_4 metabolite
Microsomal activation	1.81 ± 0.13	1.75×10^6
Microsomal activation (33 mM CCl_4)	4302 ± 300	7.5×10^2
Chemical activation	826 ± 250	3.84×10^3

[a] Anaerobic mixtures containing microsomes, NADPH, and mouse liver DNA were incubated for 30 min at 370°C with 10 ul of an ethanol solution of $^{14}CCl_4$ (27 mCi/mmol) at a concentration of 13.63 uCi/ml. DNA was isolated and counted. In the second experiment on the microsomal activation, the incubation system was as before except that cold CCl_4 was added (final concentration, 33 mM) and specific activity was 0.0137 mCi/mmol. When chemical activation was studied DNA/acetyltrimethyl ammonium bromide salts (3 mg) were heated for 16 hr at 80°C in a sealed vial under N_2 atmosphere with 3 ml of an alcoholic solution of $^{14}CCl_4$ (1.82 uCi/ml) in the presence of benzoyl peroxide. DNA was isolated and counted.

[b] All results are the mean of triplicate simultaneous experiments.

Adapted from Diaz Gomez and Castro (1980).

TABLE VII-3 Covalent Binding of ^{14}C from $^{14}CCl_4$ to Nuclear Protein Fractions From Rat Liver[a]

Nuclear Protein Fraction	^{14}C from $^{14}CCl_4$ in protein[b] (pmol/mg)	Percentage total of label in each fraction
I	4.9 - 7.5	18.25 - 19.95
II	17.9 - 10.1	29.73 - 23.61
III	8.8 - 5.3	7.76 - 5.33
IV	68.1 - 52.6	12.14 - 8.23
V	39.3 - 28.5	6.55 - 4.85
Histones	43.6 - 56.8	7.13 - 14.07
Residual	45.7 - 63.5	18.44 - 23.96

[a] $^{14}CCl_4$ administration and time of treatment as in Table VII-1. Isolated nuclear proteins were dissolved in formic acid and counted. Fraction I and II correspond to nuclear sap proteins. Fraction III and IV are deoxuribonucleoproteins. Fraction V is acid ribonucleoprotein.

[b] Pooled livers from 4 rats were used in each experiment. These results are 2 separate experiments .

Adapted from Diaz Gomez and Castro (1980).

TABLE VII-4 Covalent Binding of ^{14}C from $^{14}CCl_4$ to Different Lipid Fraction From Rat Liver[a]

Lipid Fraction	Percentage of total label in each fraction[b]
Phospholipids	75.69 - 65.11
Diglycerides	16.73 - 24.07
Cholesterol esters	0.36 - 0.24
Triglycerides	0.22 - 0.37
Free fatty acids	1.19 - 0.64
Cholesterol	5.70 - 9.50
Undetermined or loss	0.11 - 0.07

[a] $^{14}CCl_4$ administration as in Table VII-1. Animals were sacrificed 3 hr after $^{14}CCl_4$ administration. Total lipid fractions were used for counting. Covalent binding to total nuclear lipids was 113.5 pmol/mg.

[b] Pooled livers from 4 rats were used in each experiment. These results are two separate experiments.

Adapted from Diaz Gomez and Castro (1980).

is presented below, followed by a discussion of the evidence that it's biochemical sequence results in the hepatic lesions associated with carbon tetrachloride poisoning.

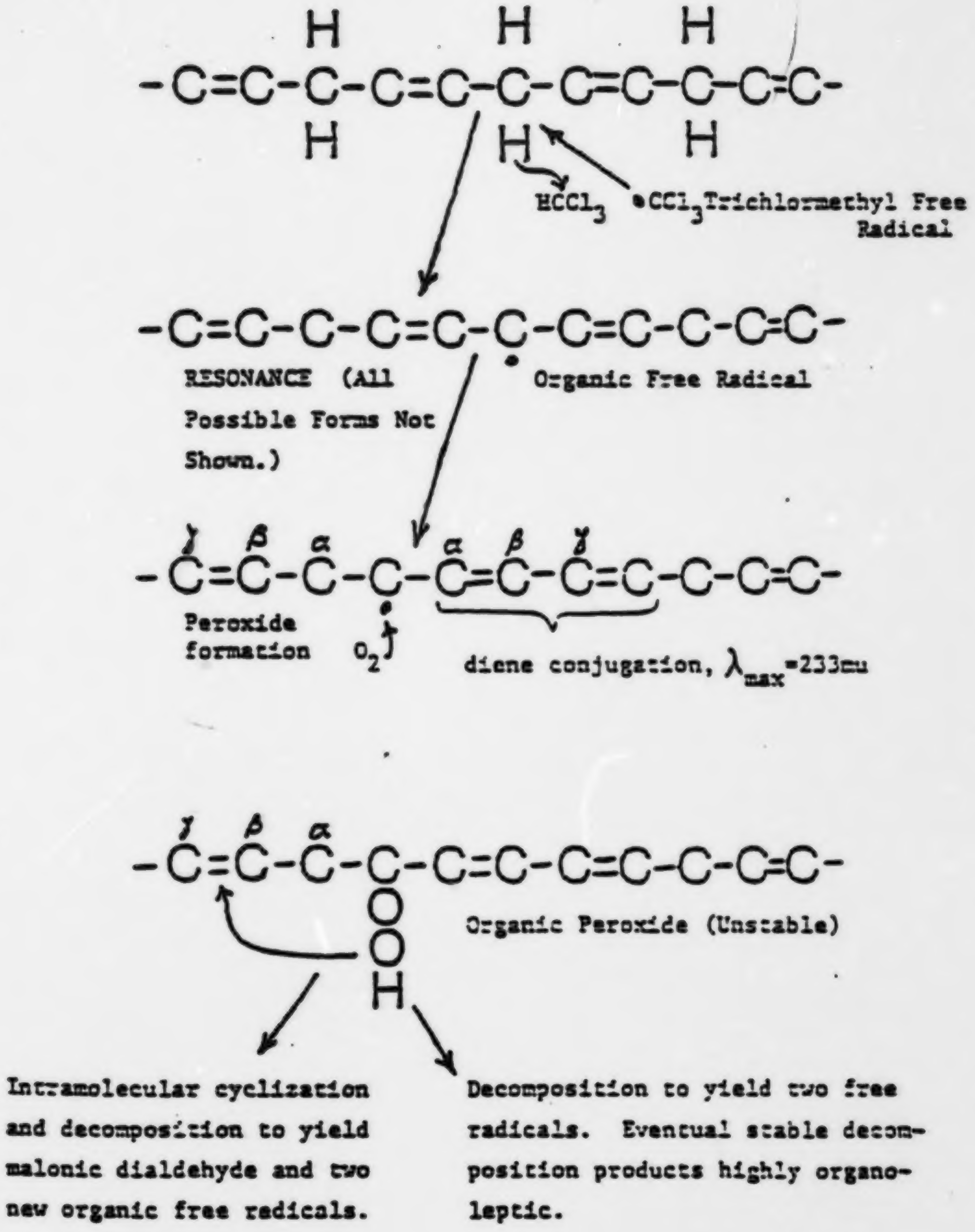
The first step in the reaction sequence proposed for lipid peroxidation is the production of free radicals, especially the trichloromethyl free radical. The radical initiates a chain reaction by reacting with the hydrogen atom of a $-CH_2$-group in an unsaturated fatty acid, generating a fatty acid free radical. On reaction with molecular oxygen, the fatty acid-free radical is converted into an unstable organic peroxide. The peroxide disintegrates in two fashions: (i) intramolecular cyclization to form malonic dialdehyde and two new free radicals, or (ii) simple homolytic fission that also yields two free radicals. This whole process occurs autocatalytically: each free radical gives rise to two new free radicals. Figure VII-1 summarizes this hypothesis (Rechnagel and Glende, 1973).

A number of indicators have been used in in vivo and in vitro assays of lipid peroxidation: pentane and ethane levels in exhaled air (arising from fatty acid decomposition) and malonic dialdehyde concentrations in hepatocytes (arising from intramolecular cyclization). Pentane production in male rats increased by factors of 4.6, 13.2, and 26.4 over that in mineral oil controls within 30 minutes following intraperitoneal administration of carbon tetrachloride doses of 160, 480, and 1,440 mg/kg bw, respectively (Sagai and Tappel, 1979).

A mechanism for the pathogenesis of carbon tetrachlorideinduced hepatic lesions based on lipid peroxidation has been proposed (Pasquali-Ronchetti et al., 1980). According to this hypothesis, lipid peroxidation is suggested to affect primarily unsaturated acyl chains of membrane phospholipids, resulting in breakage of the hydrocarbon and loss of phospholipids from the membrane. Lipid peroxidation would therefore produce progressive degenerative changes in the assembly of membranous structures such as microsomes and of (rat) liver endoplasmic reticulum.

This hypothesis is supported by studies showing that treatment with carbon tetrachloride produced lipid peroxidation in rat liver endoplasmic reticulum at a concentration of 0.5 ml/100 g bw (Pasquali-Ronchetti et al., 1980); caused disintegration of endoplasmic reticulum in vitro within 10 minutes at a concentration of 636 mg/liter (Pasquali-Ronchetti et al., 1980); and was incorporated predominantly into liver phospholipids in rats (Table VII-5) (Ciccoli et al., 1978).

Two pieces of contrary evidence have been presented by Diaz Gomez, et al. (1975). One was that the order of species susceptibility to liver necrosis from carbon tetrachloride more closely parallels the species order for [^{14}C] carbon tetrachloride binding to cellular components than the species order for lipid peroxidation:


HCCl3
•CCl3 Trichlormethyl Free Radical
RESONANCE (All Possible Forms Not Shown.)
Organic Free Radical
Peroxide formation
O2
diene conjugation, λmax=233mu
Organic Peroxide (Unstable)
γ β α
α β γ

Intramolecular cyclization and decomposition to yield malonic dialdehyde and two new organic free radicals.

Decomposition to yield two free radicals. Eventual stable decomposition products highly organoleptic.

Figure VII-1. Free Radical Initiated, Autocatalytic Peroxidation of Polyenoic Long-Chain Fatty Acids (Adapted from Recknagel and Glende 1973).

<Most susceptible	Lease suceptible>
Liver necrosis	mouse> guinea pig = hamster> rat> chicken
$[^{14}C]CCl_4$ binding	mouse = hamster> guinea pig> chicken = mouse
Lipid peroxidation	rat> hamster = guinea pig> chicken = mouse

A second result of their experiments was that for mice, necrosis proceeded for 24 hours in the absence of lipid peroxidation. Together, these pieces of evidence imply that caution is required in accepting the lipid peroxidation mechanism for carbon tetrachloride toxicity.

Summary

The metabolism of carbon tetrachloride is thought to proceed through the formation of the trichloromethyl free radical. The hepatotoxicity of carbon tetrachloride has been attributed to subsequent lipid peroxidation initiated by this radical in the following manner: a hydrogen atom is abstracted by the free trichloromethly radical from a long-chain fatty acid to form chloroform and a fatty acid-free radical. Molecular oxygen, because of its triplet ground state, binds with the unparied electron on the fatty acid radical to form an organic peroxide. The peroxide is unstable and decomposes to form more organic-free radicals, which in turn form more organic peroxides. This process appears to lead to fatty acid chain decomposition, with the resulting breakdown of membrane structure. This breakdown may lead to a halt in lipid excretion via the Golgi apparatus, with fatty liver occurring as a consequence. Cell necrosis would also follow directly from lipid destruction. The mechanism by which lipid peroxidation could lead to cell transformation is not explained yet, and the molecular events leading to carbon tetrachloride carcinogenicity remain unknown.

In addition to this proposed lipid peroxidation mechanism, which produces chloroform, two minor metabolic pathways have been postulated: dimerization to two trichloromethyl free radicals to form hexachloroethane and the formation of a trichloromethyl peroxy radical which may result in production of phosgene and carbon dioxide. Both hexachloroethane and phosgene are toxic, but the extent of their contribution to observed hepatotoxicity is unknown. A fourth possible mechanism of hepatotoxicity is the binding of trichloromethyl radical to cellular proteins.

VIII. RISK ASSESSMENT

The possibility that carbon tetrachloride is carcinogenic in humans has been a subject of concern, and has resulted in the development of several human risk assessments. This section of the report summarizes carcinogenic risk assessments prepared by the National Academy of Sciences (NAS) and the U.S. Environmental Protection Agency (EPA).

Current methods used to estimate carcinogenic risk have in common the assumption that there is no threshold level for the action of a carcinogen. The state-of-the-art and data now available are such that no one method can accurarely predict and/or model the absolute numbers of excess cancer deaths attributable to carbon tetrachloride in drinking water. Because of biological variability and the assumptions used, each of the methods used to quantify carcinogenic risk leads to a different value. The estimates and their interpretation may vary widely. In addition, none of the methods now used to quantify carcinogenic risk can account for the increased risk of carbon tetrachloride exposure to sensitive populations.

Quantification of Carcinogenic Risk

Because of positive results in animal carcinogenicity studies, carbon tetrachloride can be considered a suspect human carcinogen. Data from these animal studies have been used by the NAS and EPA's Carcinogen Assessment Group (CAG) to calculate the number of additional cancer cases that may occur when carbon tetrachloride is consumed in drinking water over a 70-year lifetime. The results of these calculations are shown in Table VIII-1.

The criteria for the CAG (USEPA 1980c, 1983) and NAS (1977) risk calculations differ in several respects: (1) NAS used the multistage model, while CAG used an "improved" multistage model. (2) NAS used the data set from the National Cancer Institute (NCI) negative study in male rats while CAG used the data set from NCI's positive study in male mice. Because of these differences, the carbon tetrachloride levels reported by CAG for cancer risk levels are approximately 1/10th those reported by NAS.

The U.S. Environmental Protection Agency has also developed Ambient Water Quality Criteria based on estimates of the increased lifetime cancer risk resulting from a lifetime consumption of both drinking water (2 liters per day) and aquatic life (6.5 g of fish and shellfish per day). These risk estimates differ from those in the previous risk assessments, which are based on consumption of drinking water alone. These criteria for lifetime cancer risks of 10^{-5}, and 10^{-6}, and 10^{-7} are 4.0, 0.40, and 0.04 ug/liter, respectively (USEPA 1980a).

Sensitive Populations

Sensitive populations are subgroups within the general population which appear at higher than average risk upon exposure to carbon tetrachloride. Some of the populations that may be

Table VIII-1 Estimates of Additional Carcinogenic Risk Following Exposure of Humans to Carbon Tetrachloride in Drinking Water[a]

Excess cancer risk/lifetime	Carbon tetrachloride concentrations (ug/liter)		
	CAG (upper 95% confidence limit)	NAS (upper 95% confidence limit)	NAS (point estimate) (95% confidence range)
10^{-4}	42.2	450	1100-900
10^{-5}	4.2	45	110-90
10^{-6}	0.4	4.5	11-9

[a] An average daily drinking water consumption of 2 liters per day was assumed.

at greater risk include human fetuses, alcohol consumers, and males of reproductive age. This section will deal with the possible effects of age, sex, and nutritional status on toxicity of carbon tetrachloride.

The studies of Reuber and Glover (1968) with rats suggest that sensitivity to the effects of carbon tetrachloride may vary with age and sex. In these studies, inbred Buffalo rats 4, 12, 24, or 52 weeks of age were injected subcutaneously twice weekly for 12 weeks with carbon tetrachloride (1000 mg/kg bw in corn oil). Cirrhosis of the liver following exposure increased with age in male rats, whereas female sensitivity increased up to 24 weeks and declined thereafter. Female sensitivity exceeded male sensitivity in the 4- and 12-week groups; this pattern reversed after 24 weeks.

Nutritional status may also affect the degree of toxicity following exposure to carbon tetrachloride in rats. Gyorgy et al. (1946) exposed young rats on various diets to approximately 300 ppm of carbon tetrachloride in a gas chamber 7 hours per day, 5 days per week for 5 months. Animals were then sacrificed and histopathological effects on the liver and kidneys were determined. Compared to these signs of toxicity in animals fed standard chow, these effects were more severe in animals fed a diet high in lipid and low in carbohydrate, or a diet low in protein. Methionine appeared to protect against the increased toxicity (particularly kidney damage) caused by low-protein diets.

Interaction of Carbon Tetrachloride with Other Chemicals

The interaction of carbon tetrachloride with certain chemicals has resulted in an enhancement of the toxic effects produced in animals by either chemical alone. Exposure of animals to certain environmental carcinogens in combination with carbon tetrachloride has resulted in an increase in carcinogenic efficacy. In addition, certain chemicals appear to increase the toxic effects of carbon tetrachloride on the liver and other organs of experimental animals. There is also clinical evidence that two of these chemicals, isopropanol and ethanol, may potentiate carbon tetrachloride toxicity in humans (see Section VII).

Carcinogenic Effects. The interaction of carbon tetrachloride with several carcinogens has been studied. The effects of carbon tetrachloride on dimethylnitrosamine-induced carcinogenicity were studied in male Sprague-Dawley rats treated with a single dose of carbon tetrachloride (4,000 mg/kg bw) by gavage 42 or 60 hours prior to a single intraperitoneal dose of dimethylnitrosamine (20 or 40 mg/kg bw). This treatment resulted in a greater than additive increase in incidence of tumors or tumor-like lesions of the liver and kidney at 12 months as compared to rats treated with carbon tetrachloride or dimethylnitrosamine along (Pound et al., 1973). If the pretreatment took place more than 60 hours before injection of dimethylnitrosamine, the incidence of kidney neoplasms decreased, while that of liver neoplasms increased further.

Similar results were obtained with N-butylnitrosurea (Takizawa et al., 1975). Experimental male ICR/ICL mice were treated with 80 mg of carbon tetrachloride subcutaneously 1 day before administration of 10 or 20 mg of N-butylnitrosurea in 50% ethanol by gavage. This treatment resulted in the induction of hepatomas after 15 months in 12 of 28 mice (dose groups combined) as compared to 1 of 18 mice given N-butylnitrosurea but not carbon tetrachloride. However, since the ethanol vehicle was not given to all controls receiving carbon tetrachloride alone, the possible ethanol potentiation of carbon tetrachloride effects could not be fully evaluated (see the following discussion of ethanol effects on carbon tetrachloride toxicity).

The effects of carbon tetrachloride on the carcinogenicity of 2,7-bis-(acetoamido)fluorene (2,7-AAF) were also studied. Carbon tetrachloride (1,4000 mg in corn oil) was given by gavage once weekly for 8 weeks to male SMA/Ms mice fed chow containing 0.025% of 2,7-AAF. A greater than additive effect on the incidence of hepatomas was observed in these animals as compared to controls, which received carbon tetrachloride or 2,7-AAF for 8 weeks, or carbon tetrachloride for one 8-week period and 2,7-AAF for another 8-233k period (Kozuka and Sassa, 1976).

Toxicologic Effects. Many chemicals have been reported to potentiate noncarcinogenic effects of carbon tetrachloride. Hewitt et al. (1980), in their review of studies on potentiation of hepatotoxic effects of carbon tetrachloride by various agents, have postulated that ketones (e.g., acetone, methyl ethyl ketone) or chemicals that can be metabolized to ketones (e.g., isopropanol, 2-butanol, 1,3-butanediol) potentiate the effects of carbon tetrachloride and other haloalkanes.

Traiger and Plaa (1971) administered isopropanol (2,000 mg/kg bw by gavage) to male Sprague-Dawley rats 18 hours before intraperitoneal injection with carbon tetrachloride (160 mg/kg bw in corn oil). The SGPT activity of these animals was 22 times higher than that in controls receiving carbon tetrachloride alone. In addition, SGPT activities in animals receiving isopropanol and carbon tetrachloride at 160 mg/kg bw were higher than those in animals receiving carbon tetrachloride at 1,600 mg/kg bw without isopropanol.

Later studies on isopropanol potentiation (reviewed by Hewitt et al., 1980) using an inhibitor of isopropanol metabolism demonstrated that potentiation probably depended on the metabolism of isopropanol to acetone. When the blood concentration of isopropanol was kept high and that of acetone was low, the potentiating capacity of isopropanol was significantly reduced. Acetone administered directly (2,000 mg/kg bw) potentiated the hepatotoxic effects of carbon tetrachloride (160 mg/kg bw) given 18 hours later. Therefore, acetone rather than isopropanol appeared to be responsible for the potentiation observed.

Isopropanol was also implicated as the potentiating factor in carbon tetrachloride toxicity (renal and hepatic) in humans after accidental exposure to carbon tetrachloride in an isopropanol packaging plan (Folland et al., 1976). Elevated levels of acetone were found in samples of the expired air of these workers.

Traiger and Bruckner (1976) studied the effects of 2-butanol and its ketone metabolite 2-butone (methyl ethyl ketone) on the potentiation of carbon tetrachloride hepatotoxicity. 2-Butanol (1,800 mg/kg bw) or 2-butanone (1,500 mg/kg bw) were administered orally to male Sprague-Dawley rats 16 hours prior to intraperitoneal injection of carbon tetrachloride (160 mg/kg bw in corn oil). SGPT activity 24 hours after carbon tetrachloride exposure was 30-fold higher in both groups than in controls given carbon tetrachloride alone. Glucose-6-phosphatase activity decreased and triglyceride levels increasd in experimental animals as compared to controls. 2-Butanol had an apparent oral half-life of 2.5 hours; by the time carbon tetrachloride was administered, most of the 2-butanol present had been metabolized to 2-butanone or eliminated. The dose of 2-butanone used approximated the level of 2-butanone in the blood after administration of 2-butanol. Because the effect of both treatments were similar, the authors proposed that 2-butanone was responsible for carbon tetrachloride potentiation in both treatments. Therefore, a similar mechanism was implicated for 2-butanol as for isopropanol: metabolism to the ketone.

Administration of 1,3-butanediol to rats has been associated with a rapid rise in the blood concentration of ketone bodies. This compound was therefore used to test the hypothesis that matabolic ketosis results in an increase in carbon tetrachloride hepatotoxicity (Hewitt and Plaa, 1979). Male Sprague-Dawley rats received 1,3-butanediol (5 g/kg bw) three times daily for 3 days. On day 3, the rats received an intraperiotoneal dose of carbon tetrachloride (160 mg/kg bw in corn oil), and were sacrificed 24 hours later. SGPT activity in experimental animals incrased 18-fold over that in controls receiving carbon tetrachloride alone, while hepatic glucose-6-phosphatase activity in experimental animals decreased compared to controls. These results, therefore, also implicated ketone's in the potentiation of carbon tetrachloride toxicity.

Several other chemicals that potentiate carbon tetrachloride toxicity, although complex in structure, also contain ketone groups (i.e., phenobarbital, pentobarbital, triamcinolone, progesterone, and Kepone). Phenobarbital has been demonstrated to markedly enhance carbon tetrachloride liver damage in mice and rats. Gans et al. (1976) detected an increase in liver weight, protein synthesis, and DNA synthesis in male Swiss mice given both phenobarbital and carbon tetrachloride as compared to those given either compound alone. Tuchweber and Kovacs (1971) found that pretreatment of female ARS/Sprague-Dawley rats with phenobarbital resulted in increased mortality, liver damage, and triglyceride accumulation after carbon tetrachloride exposure as compared to animals given carbon tetrachloride alone. Pani et al. (1973) also demonstrated an increase in mortality of male Wistar rats given phenobarbital in conjunction with carbon tetrachloride as compared to those given carbon tetrachloride alone. In addition, serum enzyme activities were increased in animals given both phenobarbital and carbon tetrachloride compared to those given only carbon tetrachloride. Lindstrom and Anders (1978) detected an increase in diene conjugation in hepatic microsomal lipids in male Sprague-Dawley rats after administration of phenobarbital and carbon tetrachloride compared to that in controls receiving carbon tetrachloride or phenobarbital alone. In further studies, isolated rat hepatocytes were prepared from male Sprague-Dawley rats that had been treated with phenobarbital; control hepatocytes were prepared from untreated rats (Lindstrom et al., 1978). In cultures containing carbon tetrachloride, release of lactic dehydrogenase was potentiated in hepatocytes from treated animals compared to controls. Chang-Tsui and Ho (1980), in an experiment with pentobarbital, demonstrated potentiation of carbon tetrachloride toxicity in male ICR mice with increases in SGPT and SGOT following carbon tetrachloride exposure.

Triamcinolone and progesterone have also been shown to potentiate carbon tetrachloride hepatotoxicity (Tuchweber and Kovacs 1971). Female ARS/Sprague-Dawley rats were treated orally twice daily with 5 mg of progesterone or 2 mg of triamcinolone. After 4 days, the animals received carbon tetrachloride at either 16 or 4,000 mg/kg bw. These treatments resulted in increases in mortality, liver damage, and triglyceride accumulation in comparison to controls given only carbon tetrachloride.

Another ketone, Kepone, also appears to potentiate the hepatotoxic effects of carbon tetrachloride (Curtis et al., 1979). After being fed chow containing 0-10 ppm of Kepone for 15 days, male Sprague-Dawley rats received a single intraperitoneal dose of carbon tetrachloride (0-320 mg/kg bw in corn oil). Compared to controls given carbon tetrachloride but no Kepone, Kepone-fed animals showed increased impairment of biliary excretion at carbon tetrachloride doses of 80 mg/kg bw or above, elevated SGPT and SGOT (carbon tetrachloride doses of 160 mg/kg bw and above), and morphological changes in the (carbon tetrachloride doses of 80 mg/kg bw and higher). Some animals in these studies were also exposed to pentobarbital during anesthesia and possibly to acetone residue in the food, since acetone was used as a vehicle for

Kepone. The possible effects of these exposures on the results could not be determined. Hewitt et al. (1980) reported that Kepone potentiated the toxic effects of chloroform, a compound similar to carbon tetrachloride (i.e., 1 a haloalkane).

In addition to the alcohols previously discussed, ethanol and methanol have been shown to potentiate the effects of carbon tetrachloride. In one study, hepatotoxic effects of carbon tetrachloride were potentiated by ethanol and methanol in male Swiss-Webster mice and by ethanol in male Sprague-Dawley rats (Traiger and Plaa, 1971). SGPT activity was increased in mice exposed to both ethanol and carbon tetrachloride compared to controls receiving carbon tetrachloride alone. Rats treated with both ethanol and carbon tetrachloride also showed increased levels of hepatic triglycerides and decreased activity of hepatic glucose-6-phosphatase. Cornish and Adefuin (1966) found that a single oral dose of ethanol followed by exposure to carbon tetrachloride vapors resulted in increased levels of SGPT, SGOT, and serum isocitric dehydrogenase in male Sprague-Dawley rats. Control animals receiving carbon tetrachloride or ethanol alone had lower levels of these enzymes, although some effect occurred with carbon tetrachloride alone. Maling, et al. (1975) also found an increase in SGPT activity in male Sprague-Dawley rats treated both with ethanol and carbon tetrachloride compared to rats treated with carbon tetrachloride alone. Increases were also noted in liver triglycerides, hepatic necrosis, and the covalent binding of carbon tetrachloride to liver protein and lipid.

Strubelt et al. (1978) also reported increases in serum enzyme activities and changes in liver morphology in male Wistar rats exposed to ethanol plus carbon tetrachloride compared to controls given either chemical alone. Cantilena et al. (1979) demonstrated the methanol potentiation of carbon tetrachloride hepatoxicity in male Sprague-Darley rats. Plasma alanine aminotransferase activity and hepatic triglyceride levels were increased and hepatic glucose-6-phosphotase activity was decreased compared to controls given carbon tetrachloride alone.

Ethanol and methanol have been judged to be less effective than isopropanol in potentiating the toxic effects of carbon tetrachloride in mice and rats (Traiger and Plaa, 1971). In humans, the effects of potentiation of ethanol and methanol on carbon tetrachloride toxicity have not been quantified. Nevertheless, case reports appear to indicate ethanol potentiating effects in humans qualitatively similar to those in animals.

Another class of chemicals which have been reported to interact with carbon tetrachloride to produce toxic effects is polychlorinated biphenyls. Carlson (1975) reported the potentiation of carbon tetrachloride hepatotoxicity in male albino rats by the polychlorinated biphenyls Arochlor 1254, 1221, and 1260. The animals received intraperitoneal injections of one of these compounds in corn oil for 6 days. Twenty-four hours after the last injection, the rats were exposed to carbon tetrachloride vapors (3,700-26,400 mg/m^3; 590-4,200 ppm) for 2 hours. Changes were demonstrated in various serum and liver enzyme activities after these treatments as compared to activities after carbon tetrachloride treatment or Arochlor treatment alone.

Summary

Carbon tetrachloride is a known animal carcinogen and a suspect human carcinogen. The risk of cancer from human exposure to carbon tetrachloride in drinking water has been estimated by the NAS and the CAG. These quantification efforts differed in methodology and in animal data used. An excess cancer risk of 10^{-5} over a 70-year lifetime would result from exposure to drinking water containing tetrachloride at 4.2 ug/liter in the CAG estimate or 45 ug/liter in the NAS estimate.

In addition to the quantitative estimation of risk, two factors that can be considered in evaluating risk of exposure to a chemical are the variation in sensitivity among populations and the interaction of the chemical with others. In the case of carbon tetrachloride, studies in rats have suggested that young females (less than 12 weeks old) are more sensitive to the chemical's cirrhotic effects, with the sensitivity pattern reversing at older ages. Furthermore, dietary changes (high-lipid/low-carbohydrate diets, or low protein diets) were reported to render rats more sensitive to liver and kidney damage following carbon tetrachloride exposure. These conclusions can be only tentative.

Greater than additive toxicity resulting from exposure to carbon tetrachloride concomitantly with other chemicals has been reported in many animal studies. The interactions have been of two forms.

1) Enhanced carcinogencity when carbon tetrachloride was given before a carcinogen. Greater than additive numbers of liver and kidney tumors were seen when dimethylnitrosamine, n-butylurea, or 2-AAF was given after carbon tetrachloride.

2) Enhanced hepatotoxic effects when carbon tetrachloride was given along with a second chemical, usually a ketone or an alcohol that is metabolized to a ketone. Potentiating effects on carbon tetrachloride toxicity have been best documented for isopropanol and butanol. Kinetic and metabolic inhibition studies have been consistent with the hypothesis that these alcohols must be metabolized to ketones (acetone and methyl ethyl ketone, respectively) before they exert their potentiating effects. Among the other alcohols reported to potentiate carbon tetrachloride toxicity are 1,3-butanediol (which is associated with a rapid rise in ketone bodies), ethanol, and methanol. In addition, potentiating effects have been reported for several chemicals with ketone groups (phenobarbital, pentobarbital, triamcinolone, progesterone, and Kepone), and for several polychlorinated biphenyls.

IX. QUANTIFICATION OF TOXICOLOGICAL EFFECTS OF CARBON TETRACHLORIDE

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. From this an Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{(\text{NOAEL or LOAEL in mg/kg/day})\ (\text{Body weight in kg})}{\text{Uncertainty/Safety factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in L/day}} = \text{mg/L}$$

where:

NOAEL = no-observed-adverse-effect level.

LOAEL = lowest-obvserved-adverse-effect level.

Body weight = 70 kg for adult or 10 kg for child.

Drinking Water volume = 2 L per day for adults or 1 L per day for children.

Uncertainty/Safety factor = 10, 100 or 1,000.

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A one-day HA for 10-kg child.
2. A one-day HA for 70-kg adult.
3. A ten-day HA for 10-kg child.
4. A ten-day HA for 70-kg adult.
5. A lifetime AADI for a 70-kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. A modification of the guidelines set forth by the National Academy of Sciences (NAS 1977, 1980) are used in establishing uncertainty factors as follows:

- o An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- o An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- o An uncertainty factor of 1,000 is used when limited or incomplete acute or chronic toxicity data in all species are available or when the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species, but human data are not available.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (i.e., carbon tetrachloride), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical via drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans from these data, it must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 liters of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The esitmates of low doses for these models can differ by several orders of magnitude. The multistage model does not necessarily give the highest or lowest risk estimates at low doses. Whether it is the most conservative, least conservative or predicts a risk in the middle of the range of risks predicted by other models is chemical specific. The multistage model is used because CAG and NAS use it.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Noncarcinogenic Effects

Varying degrees of carbon tetrachloride (CCl_4)-induced toxicity have been reported in humans and animals following acute and chronic exposures via ingestion, inhalation or dermal administration. The following paragraphs discuss the results of pertinent studies to be considered for the derivation of the RMCL at a later date.

Effects of acute exposure to low levels of CCl_4 in rats were reported by Korsrud et al. (1972). Male rats (260-400 g) were administreed single oral doses of CCl_4 (0 to 4,000 mg/kg bw) in corn oil. The rats were fasted for 6 hours before dosing and for 18 hours afterward, and then sacrificed. Assays included liver weight and fat content, serum urea and arginine levels, and levels of nine serum enzymes, produced mainly in the liver. At 20 mg/kg bw, there was histopathologic evidence of toxic effects on the liver. At 40 mg/kg bw, liver fat, liver weight, serum urea, serum arginine, and levels of six of the nine liver enzymes were increased. At higher doses the remaining three enzyme levels were also elevated. The histologic changes seen at the minimum effect level, 20 mg/kg bw, included a loss of basophilic stippling, a few swollen cells, and minimal cytoplasmic vacuolation. This study was used by EPA's Office of Drinking Water to derive the existing one-day and ten-day health advisories for CCl_4 (USEPA, 1981d).

Murphy and Malley (1969) investigated the effects of single oral doses of CCl_4 on the corticosterone-inducible liver enzymes, tyrosine-α-ketoglutarate transaminase, alkaline phosphatase, and tryptophan pyrrolase in rats. Specifically, groups of 4-7 male rats were administered by gavage 400, 800, 1600, 2400, or 3200 mg/kg undiluted CCl_4. Single doses of 400 mg/kg or greater of CCl_4 increased liver tyrosine-α-ketoglutarate transaminase acid and alkaline phosphatase, but not tryptophan pyrrolase activity within 5 hours. The National Academy of Sciences (NAS) used this experiment to calculate one-day and seven-day suggested no-adverse-response levels (SNARLs) for CCl_4 (NAS, 1980).

In a chronic oral exposure study (Alumot et al., 1976), groups of 36 rats (18 males and 18 females) were fed mash containing CCl_4 at 0, 80, or 200 mg/kg of feed. The authors calculated that the 200 mg/kg of feed represented a daily dose of 10-18 mg/kg bw. After 2 years, the surviving animals were sacrificed. Serum values for glucose, protein, albumin, urea, uric acid, cholesterol, SGOT, and SGPT in the treated animals did not differ from those in controls. No fatty livers were detected in the treated animals. The authors found no biochemical changes attributable to CCl_4 exposure. However, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals. At 18 months, the survival ranged from 61-89%, and more than half the animals were dead at 21 months. Although the authors indicated that 10-18 mg/kg bw (200 mg/kg of feed) is a no-adverse-effect level of CCl_4 over 2 years, this conclusion may be questioned because of the poor survival and chronic respiratory infection of experimental animals.

Recently, Bruckner et al. (manuscript in preparation) investigated the oral toxicity of CCl_4 in male Sprague Dawley rats. In Study I, rats weighing 300-350 g were randomly divided into groups of 5 animals each. The animals were administered by gavage 0, 20, 40, or 80 mg CCl_4/kg bw (in corn oil) daily for 5 days, allowed 2 day without dosing, and then dosed once daily for 4 additional days. One group of animals at each dosage level was sacrificed at 1, 4, and 11 days following the initiation of the dosing. The following observations were made: (1) One-day treatment: At 20 and 40 mg/kg, there were no significant changes in blood urea nitrogen (BUN), glutamic-pyruvic transaminase (GPT) activity, sorbitol dehydrogenase (SDH) activity, ornithine-carbamyl tranferase (OCT) activity or histopathological changes in the liver or kidneys. At 80 mg/kg, increased GPT ($p<0.05$) activity was observed. Furthermore, there was vacuolization of centrilobular cells adjacent to the central vein in each liver. (2) Four-day treatment: Animals exposed to 20 mg/kg did not exhibit any significant alterations in enzymatic activities. At 40 and 80 mg/kg, there was an increase ($p<0.05$) in SDH activity. GPT activity was elevated ($p<0.05$) in rats given 80 mg/kg. Centrilobular vacuolization was noted in groups receiving 20 and 40 mg/kg. Midzonal vacuolization was observed in rats exposed to 80 mg/kg. (3) Eleven-day treatment: The lowest dose (20 mg/kg) produced an increase

($p<0.01$) in SDH activity whereas 40 and 80 mg/kg increased ($p<0.05$ and <0.001) serum levels of all three enzymes. The extent of morphological changes in the liver was dose-related.

In Study II, rats weighing 200-250 g were randomly divided into groups of 15 to 16 animals each. The animals were given by gavage 0, 1, 10, or 33 mg CCl_4/kg bw (in corn oil). The animals were dosed on a daily basis, 5 times weekly, for a total period of 12 weeks. Blood samples were obtained from alternate animals at the following intervals: 2, 4, 6, 8, 10, and 12 weeks post-treatment. The serum was analyzed for BUN, GPT, SDH and OCT. At 1 mg/kg, there were no significant biochemical/histopathological changes at any time during the study. SDH, the most sensitive index of hepatotoxicity, was elevated ($p<0.05$) in rats receiving 10 mg/kg throughout the study. Also, these rats exhibited mild hepatic centrilobular vacuolization. At 33 mg/kg, levels of GPT, SDH and OCT were markedly increased ($p<0.01$) and severe hepatic lesions were apparent. Prominent fibrosis, bile duct hyperplasia, and hepatocellular vacuolization were seen in the portal and periportal regions of hepatic lobules. Nuclear pleomorphic and severe cytoplasmic degenerative changes were commonly present in mid- and centrilobular hepatocytes. There was no evidence that CCl_4 was nephrotoxic. Studies I and II will be used for the derivation of one-day and ten-day Health Advisories; and lifetime AADI.

A cross-sectional epidemiologic study (Sonich et al., unpublished) examined health effects of CCl_4 ingestion in humans. Seventy tons of CCl_4 were spilled in the Kanawha and Ohio Rivers in 1977. Measurements of raw water revealed maximum concentrations of 0.340 mg/l. Twenty-one cities situated along the river were involved in the study. These cities represented areas that obtained their drinking water directly from the river and/or areas that obtained their drinking water from sources not influenced by the quality of the river water. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to CCl_4 were estimated for each city along the river. The results of routine tests measuring serum chemistries reflecting liver and kidney function along with basic epidemiologic information were abstracted from approximated 6,000 medical records. The results obtained for serum creatinine show a positive and statistically significant ($p<0.05$) relationship between the CCl_4 exposure and the frequency of elevated levels of serum creatinine in exposed patients. No similar results were found for the other parameters analyzed.

Stewart et al. (1961) reported the toxic effects of experimental exposure of human volunteers to CCl_4 vapor. Healthy males, 30-59 years of age, were exposed to concentrations of 63, 69, and 309 mg/m^3 of CCl_4 (99% pure) in an exposure chamber for 180 minutes at the two lower doses or 70 minutes at the highest dose. All subjects had undergone periodic physical examinations;

some participated in more than one of the exposure experiments, which were conducted more than 4 weeks apart. Six subjects exposed to the highest concentration experienced no nausea or light-headedness, and CCl_4 was not detected in blood and urine during or after exposure. One of these six subjects had an increased level of urinary urobilinogen 7 days after exposure. In addition, two of four subjects exposed to the highest concentration and monitored for serum iron showed a decrease within 48 hours after exposure. CCl_4 was also not detected in the blood or urine of volunteers exposed at 63 or 69 mg/m^3, and the volunteers reported no physiologic effects. No changes in blood pressure, serum transaminase levels, or urinary urobilinogen levels were noted.

B. Quantification of Noncarcinogenic Effects

Korsrud *et al*. (1972) reported that the lowest acute oral dose of CCl_4 inducing an adverse effect in rats was 20 mg/kg bw (lowest-observed-adverse-effect level) (Korsrud *et al*., 1972). The length of this study was only 18 hours. Alumot *et al*. (1976) proposed that 10 mg CCl_4/kg is the acceptable daily intake or no-adverse-effect-level for rats of both sexes fed CCl_4 for a period of 2 years. However, as discussed earlier, this study could not be considered at this time due to high morbidity/mortality rate among experimental rats and their respective controls. It is noteworthy that the results for the first year of this study was used by ECAO-Cin. (USEPA, 1982a) to derive the ambient water level for CCl_4 of 3.3 mg/l for protection against CCl_4-induced noncarcinogenic effects. It should be noted that values for consumption of contaminated water/fish (2 l/day and 0.0065 kg/day) and bioconcentration factor for CCl_4 (18.75 l/kg) were taken into consideration during the calculation of this protective level. The corresponding "drinking water only" value is 3.5 mg/l.

EPA's existing health advisories and NAS' SNARLs for CCl_4 could be summarized as follows:

	EPA-health advisory	NAS-SNARL
One-day	0.2 mg/l	14 mg/l
Seven-day	-	2 mg/l
Ten-day	0.02 mg/l	-
Longer-term	None[a]	None[b]

[a] EPA did not calculate a longer-term health advisory for CCl_4 due to lack of acceptable chronic exposure data.

[b] NAS did not determine a longer-term SNARL for CCl_4 because this chemical is a carcinogen in animals.

The assessment of human health risks, that is, the likelihood of certain adverse effects from given exposure scenarios, is hampered by the paucity of good dose-response data in humans. A human no-observed-adverse-effect level (NOAEL) for oral ingestion was reported as 0.2 mg/day ($\approx$ 0.1

ppm) for 1-day exposure (Sonich et al., 1981, unpublished). The observed effect was a dose-related increase in the frequency of elevated serum creatinine levels in the exposed population. However, these findings have not been published at this time. A human no-observed-effect level (NOEL) for inhalation was reported as 63 mg/m^3 ($\approx$ 10 ppm) for 3-hour exposure (Stewart et al., 1961). The monitored effects were changes in serum enzyme and iron levels.

At this juncture, the two studies of Bruckner et al. manuscript in preparation provide us with acceptable data (dose-response relationship, length of exposure, etc.) to revise the existing health advisories (USEPA, 1981d) and to derive a lifetime AADI as follows:

Study I (Bruckner et al., manuscript in preparation) showed that one-day exposure to 20 or 40 mg CCl_4/kg did not produce any significant changes in BUN, GPT, SDH, OCT, or histopathological changes in the liver and kidneys. At 80 mg/kg, increased GPT activity and vacuolization of centrilobular cells adjacent to the central vein in each liver were observed. Therefore, the largest dose with no significant biochemical or histopathological effects is 40 mg CCl_4/kg (NOAEL).

One-Day Health Advisory (HA)

a. One-Day Health Advisory for Child

$$\frac{(40\ \text{mg/kg/day})\ (10\ \text{kg})}{(100)\ (1\ \text{l/day})} = 4\ \text{mg/l}$$

where: 40 mg/kg/day = NOAEL following one-day exposure

10 kg = weight of child

100 = uncertainty factor based upon a good animal study revealing NOAEL

1 l/day = assumed water consumption by a 10-kg child

b. One-day Health Advisory for Adult

$$\frac{(40\ \text{mg/kg/day})\ (70\ \text{kg})}{(100)\ (2\ \text{l/day})} = 14\ \text{mg/l}$$

where: 40 mg/kg/day = NOAEL following one-day exposure

70 kg = weight of adult human

100 = uncertainty factor based upon a good animal study revealing NOAEL

1 l/day = assumed water consumption by a 70-kg adult human

Study I (Bruckner *et al*., manuscript in preparation) found that eleven-day exposure to the lowest dose (20 mg CCl_4/kg) induced an increase in SDH whereas 40 and 80 mg CCl_4/kg increased serum levels of GPT, SDH, and OCT. The extent of morphological changes in the liver was dose-related. Thus, the lowest dose of 20 mg/kg should be considered as the NOAEL.

Ten-Day Health Advisory

a. Ten-day Health Advisory for Child

$$\frac{(20\text{ mg/kg/day})\ (5\text{ days})\ (10\text{ kg})}{(1000)\ (7\text{ days})\ (1\text{ l/day})} = 0.142\text{ mg/l or }142\text{ ug/l}$$

where: 20 mg/kg/day - NOAEL following 11-day exposure

5/7 days = fraction converting from 5 to 7-day oral exposure

10 kg = weight of child

1000 = uncertainty factor based upon a good animal study revealing NOAEL

1 l/day = assumed water consumption by a 10-kg child

b. Ten-day Health Advisory for Adult

$$\frac{(20\text{ mg/kg/day})\ (5\text{ days})\ (70\text{ kg})}{(1000)\ (7\text{ days})\ (2\text{ l/day})} = 0.5\text{ mg/l or }500\text{ ug/l}$$

where: 20 mg/kg/day = NOAEL following 11-day exposure

5/7 days = fraction converting from 5 to 7-day oral exposure

70 kg = weight of adult human

1000 = uncertainty factor based upon a good animal study revealing NOAEL

2 l/day = assumed water consumption by a 70-kg adult human

Study II (Bruckner *et al*., manuscript in preparation) showed that, following 90-day exposure to 1 mg CCl_4/kg, there were no significant biochemical/histopathological changes in rats. At 10 mg/kg, SDH, the most sensitive index of hepatotoxicity, was elevated, and mild hepatic centrilobular vacuolization was detected.

Lifetime Adjusted Acceptable Daily Intake (AADI) For Adult

$$\text{ADI} = \frac{(1\ \text{mg/kg/day})\ (5\ \text{days})\ (70\ \text{kg})}{(100)\ (10)\ (7\ \text{days})} = 0.050\ \text{mg/day or } 50\ \text{ug/day}$$

where: 1 mg/kg/day = NOAEL following 90-day exposure

5/7 days = fraction converting from 5 to 7 day oral exposure

70 kg = weight of adult human

100 = uncertainty factor based upon a good animal study revealing NOAEL

10 = uncertainty factor to take into account the length of exposure (i.e., convert 90-day to lifetime exposure)

$$\text{AADI} = \frac{50\ \text{ug/day}}{2\ \text{l/day}} = 25\ \text{ug/day}$$

where: 50 ug/day = ADI

2 l/day = assumed water consumption by a 70-kg adult human

C. Carcinogenic Effects

The carcinogenic effects of CCl_4 have been well documented. Oral administration of CCl_4 have been shown to be carcinogenic in rats, mice, and hamsters. In all three species, liver neoplasms developed although hamsters appeared to be the most sensitive.

The International Agency for Research on Cancer (IARC) concluded that the evidence from animal studies demonstrating CCl_4-induced hepatic neoplasms was sufficient to indicate experimental animal carcinogenesis (IARC, 1979). The National

Cancer Institute (NCI) also identifies CCl_4 as an animal carcinogen and has used it as the positive control in three of its bioassays. The following paragraphs will focus on pertinent studies demonstrating the carcinogenicity of CCl_4.

In an NCI (1976) bioassay for trichloroethylene, CCl_4 was used as the positive control. Rats: The positive control groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg bw for males, 80 and 159 mg/kg bw for females. The incidence of hepatocellular carcinomas was increased in animals exposed to CCl_4 as compared with pooled colony controls. However, this was statistically significant only for females given the low dose as compared with the colony controls and not the matched controls. Absolute incidence of hepatic neoplasms was low (5% in the animals exposed to CCl_4). This may be attributed to the resistance by this rat strain to CCl_4. The incidence of other neoplasms was acknowledged but not quantified. This study was used by NAS (1978) in determining the carcinogenic risk estimate for CCl_4 due to the dose levels used and the appropriate length of the study. Mice: B6C3F1 male and female mice (35 days of age, 50 per group) were given CCl_4 (1,250 or 2,500 mg/kg bw) in corn oil by gavage five times weekly for 78 weeks. Surviving mice were sacrificed at 92 weeks from the start of the study. There were 20 control mice of each sex that were given corn oil only. A necropsy was performed on all mice along with complete histological examinations.

Most male and female mice treated with CCl_4 were dead by 78 weeks. Hepatocellular carcinomas were found in practically all mice receiving CCl_4, including those dying before termination of the test. The first carcinomas were observed in low dose female mice at 16 weeks, in high dose female mice at 19 weeks, in high dose males at 26 weeks and in low dose males at 48 weeks, compared to 72 weeks for pooled control males and 90 weeks for pooled control females. Cystic endometrial hyperplasia occurred in both control and treated female mice. Thrombosis of the atrium of the heart was seen in 9 of 41 high dose female mice (22%), all of which died with carcinomas of the liver. In summary, this study found CCl_4 to be highly carcinogenic for liver in mice and is used by the World Health Organization (WHO, 1981) in ascertaining the carcinogenic risk estimates for CCl_4.

Edwards et al. (1942) investigated the carcinogenic potential of CCl_4 in mice. The mice used were inbred strain L with extremely low incidence of spontaneous hepatomas, 2.5-3.5 months or 3.5-7.5 months of age at the onset of the experiment. The number of mice varied from 8-39 per group. Carbon tetrachloride was administered in olive oil by stomach tube usually three, but occasionally two, times weekly. Each treatment consisted of 0.1 cc of a 40% solution or 0.04 ml of CCl_4. Mice were given

46 administrations of CCl_4 over a 4-month period and were sacrificed and necropsied 3-3.5 months after the last treatment. The mice varied from 8.5-14 months of age at necropsy. The liver was examined histologically.

Hepatomas developed in 34/73 mice (47%) given CCl_4. Hepatomas were observed in 7/15 younger male mice (47%), 21/39 older male mice (54%), 3/8 younger females (38%), and 3/11 older females (27%). Cirrhosis of the liver was not mentioned. Historically, the incidence of spontaneous hepatomas in strain L mice is extremely low: 2/152 (1%) in untreated mice. One of 23 untreated virgin male mice (4%) and 0 of 28 females (0%), necropsied at 15 months of age, had tumors of the liver. Tumors were not present in 22 males and 28 females 18 months of age or in 27 female breeders 12-23 months of age. One of 24 male breeders (4%) had a tumor. In summary, strain L male and female mice were highly susceptible to the induction of hepatomas by CCl_4, and male mice were slightly more susceptible than female mice.

Della Porta _et_ _al_. (1961) exposed Syrian golden hamsters to CCl_4 in order to investigate the response of this species to carcinogens that induced liver neoplasms in other species. Ten female and 10 male Syrian golden hamsters, 12 weeks old, were used. At the onset of the experiment, males weighed an average of 99 g and females weighed an average of 109 g. At the end of the experiment, the average weight was 104 g for both sexes.

The treatment consisted of weekly administration by stomach tube of a 5% solution of CCl_4 in corn oil for 30 weeks. Controls cited were historical controls kept by the investigators in the same laboratory for a lifespan. A total of 145 female and 109 male hamsters of the same strain, fed the same diet, did not develop hepatic tumors. The authors also cited controls for a different study they conducted. In this latter study, 30 female and 50 male hamsters fed the same diet but given 0.5 ml corn oil via stomach tube twice weekly for 45 weeks also did not develop hepatic tumors. During the first 7 weeks of the former experiment, 0.25 ml of the solution containing 12.5 ul CCl_4 was given each week. This dose was then reduced to 0.125 ml and contained 6.25 ul of CCl_4. After this treatment, the survivors were kept under observation for 25 additional weeks and then sacrificed. Detailed histopathological examinations of all hamsters were conducted, except for one female lost through cannabalism at the 28th week.

Weights of the hamsters varied irregularly during the period following treatment. In general, the weights increased. Females weighed an average of 114 g and males 118 g. One female died at the 10th week of treatment; three females and five males died or were sacrificed between the 17th and the 28th week. Three females died at weeks 41, 43 and 54. The surviving three females land five males were sacrificed at the end of the 55th week.

Hamsters dying during the treatment and at the 41st week manifested cirrhosis, as well as hyperplastic nodules that were two to several layers thick. The cells showed irregularities in the shape, size and staining qualities of their cytoplasm and nucleus, with an uneven distribution of glycogen.

All of the animals, five males and five females, dying or sacrificed 13-25 weeks after the end of the treatment, had one or more hepatic carcinomas (a total of 22 tumors: 12 in the females and 10 in the males). No mention was made of toxicity in these animals. Hepatic carcinomas were not found in the other animals dying before week 43.

In summary, Syrian golden hamsters appear sensitive to the carcinogenic effects of CCl_4. Although the number of animals in this study was small, the authors considered the results to be significant because the reported historical control incidence of hepatic tumors in hamsters was 0/254. Hyperplastic nodules appeared during treatment, and carcinomas appeared after CCl_4 administration had been discontinued, which suggests that the nodules or benign tumors were precursor lesions for carcinomas. It should be noted that this study is the only report found in the available literature of CCl_4 induction of tumors in hamsters.

The above studies by NCI (1976), Edwards *et al*. (1942), and Della Porta *et al*. (1961) were used by EPA's Office of Health and Environmental Assessment (OHEA) to calculate the unit risk estimates for CCl_4 (USEPA, 1983).

Although several investigators noted that toxic effects of CCl_4 are concurrent with liver tumors (hepatomas), it has not been established that tissue damage (i.e., necrotic cirrhosis) is a necessary precursor to CCl_4-induced carcinogenesis.

Despite a wealth of data on its toxic effects, there is little definitive information on its metabolism or its mode of carcinogenic action. Among reported metabolic reactions in liver are conversion to carbon dioxide, chloroform, hexachloroethane, carbonyl chloride (phosgene), and binding to lipids and proteins. Diaz Gomez and Castro (1980) reported that ^{14}C from $^{14}CCl_4$ irreversibly binds _in vivo_ to hepatic nuclear DNA from mice and rats. Also, binding of ^{14}C from $^{14}CCl_4$ to DNA was detected _in vitro_ in incubation mixtures containing microsomes and a NADPH-generating system as well as in tissue slices. Liver nuclear proteins and lipids irreversibly bind CCl_4 metabolites. The authors concluded that (a) the interaction of CCl_4 metabolites with DNA and nuclear proteins could be relevant to CCl_4-induced liver tumors and hepatoxic effects; and (b) the epigenetic mechanisms for chemical induction of cancer, not involving CCl_4-DNA interactions could also be relevant. There have been no reports of mutagenic activity associated with CCl_4 in any of the various

Salmonella (Ames) assays. However, mutagenic activity associated with CCl_4 has been observed in a eukaryotic test system using the yeast *Saccharomyces cerevisiae* (Callon *et al.*, 1980). This report has not been confirmed and should not be accepted as conclusive evidence of CCl_4 mutagenicity. Carbon tetrachloride did not cause chromosome damage (i.e., chromatid gaps, deletions, or exchanges) during an *in vitro* chromosome assay using cultured rat-liver cells (Dean and Walker, 1979). Mirsalis and Butterworth (1980) found that treatment of male rats with CCl_4 (10 or 100 mg/kg administered by gavage) produced no increase in unscheduled DNA synthesis in cultures of primary rat hepatocytes. According to the authors, this observation indicates that CCl_4 does not act through a genotoxic mechanism. Thus, the genotoxic potential of CCl_4 obviously needs further investigation.

D. Quantification of Carcinogenic Risk

Because of positive results in animal carcinogenicity studies, CCl_4 can be considered a suspect human carcinogen. Data from the animal studies have been used by NAS (1977) and OHEA (USEPA, 1980a; 1983) to calculate the upper bound on the number of additional cancer cases that may occur when CCl_4 is consumed in drinking water over a 70-year lifetime. As shown in Table IX-I, using the OHEA and NAS data, estimates of additional carcinogenic risk following the exposure of humans to CCl_4 may be derived.

Table IX-I

Estimates of Additional Carcinogenic Risk Following Exposure to CCl_4 in Drinking Water[a]

Excess Cancer Risk (Lifetime)	CCl_4 Concentrations (ug/l)[b] OHEA (USEPA, 1980a)[d]	OHEA (USEPA, 1983)[e]	NAS (1977)[c]
10^{-4}	42.2	27.0	450
10^{-5}	4.2	2.7	45
10^{-6}	0.4	0.3	4.5

[a] assuming an average daily drinking water consumption of 2 liters

[b] lower 95% confidence limit

[c] based on NCI (1976) (rats)

[d] based on NCI (1976) (mice)

[e] based on NCI (1976) (rats and mice), Edwards *et al*. (1942) (mice), and Della Porta *et al*. (1961) (hamsters)

The criteria for the OHEA and NAS risk calculations differ in several respects: (1) NAS used the multistage model, while OHEA used an "improved" multistage model, (2) NAS used the data set from the National Cancer Institute (NCI) study in male rats while OHEA used the data set from NCI's study in male mice (USEPA, 1980a), and used a geometric mean of four studies (NCI, 1976 - mice; NCI, 1976 - rats; Edwards et al., 1942 - mice; and Della Porta et al., 1961 - hamsters) (USEPA, 1983).

EPA's Ambient Water Quality Criteria for CCl_4 (USEPA, 1980a) were based on increased lifetime cancer risk estimates of 10^{-5} (4.0 ug/l), 10^{-6} (0.40 ug/l), and 10^{-7} (0.04 ug/l) calculated by OHEA. It is noteworthy that these estimates were derived by assuming a lifetime consumption of both drinking water (2 l/day) and aquatic life (6.5 g fish and shellfish/day) grown in waters containing the corresponding CCl_4 levels. Specifically, OHEA's daily CCl_4 exposure assumptions were as follows: 94% from ingesting drinking water and 6% from consuming seafood "fish factor." The corresponding "drink water only" concentrations are 4.4, 0.44, and 0.04 ug/l, respectively.

Using the same data set as OHEA and a linear multistage model, WHO (1983) derived a recommended tentative limit for CCl_4 of 3 ug/l. This level should give rise to less than 1 additional cancer per 100,000 population for a lifetime of exposure assuming a 2-liter daily consumption of drinking water.

In addition to NAS', OHEA's and WHO's estimates of additional carcinogenic risk following exposure of humans to CCl_4 in drinking water, OHEA calculated a unit risk estimate for humans from exposure to CCl_4 in water as follows: 0.37×10^{-5} for a person continuously exposed to 1 ug CCl_4 per liter of water, (USEPA, 1983). Since no single study was entirely adequate for risk assessment, this estimate is based upon the geometric mean of four studies discussed above and correspond to drinking water concentrations presented in Table IX-I. It should be noted that EPA's Science Advisory Board approved OHEA's approach for calculating unit risk estimates for CCl_4.

E. Special Considerations

It is noteworthy that in assessing CCl_4-induced toxicity, carcinogenicity or any other harmful effect, compounds that react synergistically or antagonistically with CCl_4 must be considered. Identified synergistic substances include ethanol, kepone, PCB, and PBB. Antagonistic effects have been demonstrated with such compounds as chloramphenicol and catechol.

Sensitive populations are subgroups within the general population which appear at higher than average risk upon exposure to CCl_4. Some of the populations that may be at greater risk include human fetuses, alcohol consumers, and males of reproductive age.

F. Summary

The recommended values for one-day, ten-day for both children and adult humans, the lifetime AADI for adult humans, and the estimated lifetime cancer risks are summarized in Table IX-II.

Table IX-II

Summary of Quantification of Toxicological Effects of CCl_4

	Drinking Water Concentration	
	10-kg Child	70-kg Adult
One-Day Health Advisory	4 mg/l	14 mg/l
Ten-Day Health Advisory	142 ug/l	500 ug/l
Lifetime AADI		25 ug/l
Excess Cancer Risk		
10^{-4}		27 ug/l
10^{-5}		2.7 ug/l
10^{-6}		0.3 ug/l

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